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Original Articles

ANTIBODY TITER IN ALLERGIC CHILDREN GIVEN ADENOVIRUS VACCINE

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THE adenoviruses have been shown to be a common cause of respiratory illness in children.[1-4] We feel that in many allergic children these respiratory infections trigger asthmatic attacks.

A vaccine capable of preventing infection with the adenoviruses would be highly beneficial to children in general, and to asthmatic children in particular.

With this end in mind, we administered a polyvalent formalin-inactivated vaccine (containing strains 1, 2, 3, 4, 5, and 7) prepared from lots of virus grown on monkey kidney tissue cultures, inactivated as separate strain pools, and pooled into the final six-strain vaccine.

Children attending our clinic and also private patients were injected as follows:

Group A. Nineteen children received 0.25 c.c. subcutaneously, followed by 0.5 c.c. subcutaneously in two weeks, and were bled two weeks after the second injection.

Group B. Nineteen children received 1 c.c. of vaccine subcutaneously and were bled four weeks later.

Group C. Twenty-five children received 0.1 c.c. intradermally and were bled four weeks later.

The results are shown in Tables I, II, and III and are summarized in Table IV, which lists the percentages showing greater than twofold increases in titer.

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This study was made possible by a grant from Parke, Davis & Company, Detroit, Michigan, who also supplied the vaccine.

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†Deceased.

TABLE I. CHILDREN TREATED WITH INJECTIONS OF ADENOIDAL VIRUS, 0.25 C.C., AND TWO WEEKS LATER 0.5 C.C., AND BLED FOUR WEEKS LATER

PATIENT	TYPE 1		TYPE 2		TYPE 3		TYPE 4		TYPE 5		TYPE 7	
	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.
A. G.	< 4	< 4	4	32	320	512	256	–	64	–	16	–
B. M.	4	8	512	256	< 4	< 4	> 2048	1280	< 8	< 32	< 8	< 32
B. C.	< 4	< 4	< 4	< 4	800	256	32	200	< 16	< 16	512	512
B. R.	< 4	< 4	> 2048	< 4	> 2048	> 2048	32	400	< 16	< 16	64	–
C. A.	< 4	16	> 2048	1024	4	40	< 32	128	< 32	< 4	128	256
C.	< 8	< 4	320	256	< 8	< 4	< 32	< 16	256	512	< 32	< 16
C. T.	256	512	4	16	< 4	16	< 8	200	13	64	< 8	32
D. W.	< 4	< 4	< 4	< 4	200	640	< 16	64	< 16	< 8	< 16	64
D. H. R.	4	–	512	–	256	–	< 32	–	< 32	–	< 32	–
G.	8	4	1600	> 2048	< 4	< 4	< 16	–	< 16	–	< 16	–
H.	6	8	256	256	256	512	< 16	64	640	1024	< 16	100
M.	< 4	< 4	160	128	160	512	< 16	–	< 16	–	< 16	–
M. P.	< 4	8	512	512	< 4	8	< 32	32	< 32	80	< 32	50
N.	1024	512	< 8	8	< 8	32	< 8	–	< 8	–	< 8	–
P. J.	128	128	512	512	80	256	32	128	32	256	< 16	32
S. C.	128	128	512	512	40	128	16	–	16	–	128	–
W. J.	128	160	4	16	256	200	–	256	–	< 16	–	64
W. P.	8	16	512	1024	< 4	32	512	> 2048	< 16	16	64	400
P. K.	–	4	–	1024	–	640	–	64	–	8	–	64

TABLE II. CHILDREN TREATED WITH ONE INJECTION OF ADENOIDAL VIRUS, 1 C.C., AND BLED FOUR WEEKS LATER

PATIENT	TYPE 1		TYPE 2		TYPE 3		TYPE 4		TYPE 5		TYPE 6	
	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.
A. L.	< 4	< 4	256	800	< 4	< 4	32	64	8	< 8	< 4	< 8
A. V.	64	256	512	640	< 8	< 4	64	64	16	< 16	< 8	64
A. P.	128	128	4	< 4	< 4	< 4	16	16	16	10	16	16
C. G.	< 8	< 4	< 8	< 4	< 8	8	64	32	< 8	< 8	< 8	16
C.	256	256	256	320	640	> 2048	16	8	64	16	16	32
D. R.	4	< 4	512	400	128	256	–	–	–	–	–	–
G. C.	256	–	128	–	< 4	–	–	–	–	–	–	–
H. H.	< 4	< 4	512	128	400	> 2048	–	16	–	< 16	–	64
H. W.	< 4	< 4	100	128	512	1024	200	4	< 4	< 4	< 4	32
H. G.	< 4	< 4	< 4	< 4	< 4	50	32	< 8	< 16	< 8	50	256
L. L.	< 8	< 4	< 8	< 4	< 8	< 4	25	< 32	< 8	< 32	< 8	< 32
M. G.	512	512	32	256	< 4	4	–	32	–	32	–	< 8
M. R.	400	128	100	512	< 8	< 4	20	8	1024	512	< 8	< 8
N. H.	4	–	4	–	< 4	–	16	–	1600	–	< 8	–
P. M.	< 4	< 4	< 4	< 4	–	< 4	32	16	< 8	< 16	< 8	< 16
P. W.	< 4	< 4	< 4	< 4	–	< 4	16	< 16	< 8	< 16	< 8	< 16
R. M.	80	–	512	–	800	–	16	–	256	–	8	–
S. P.	128	128	256	512	25	256	< 8	< 8	256	512	16	256
F. A.	< 8	4	512	512	< 8	8	25	16	< 8	< 8	< 8	64

TABLE III. CHILDREN INJECTED WITH ADENOIDAL VIRUS, 0.1 C.C., INTRADERMALLY, AND BLED FOUR WEEKS LATER

PATIENT	TYPE 1		TYPE 2		TYPE 3		TYPE 4		TYPE 5		TYPE 7	
	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.	PRE.	POST.
A. W.	8	64	320	256	< 4	8	< 8	< 16	8	< 16	< 8	< 16
B. R.	> 2048	16	200	512	8	512	16	256	8	32	16	32
B. A.	> 2048	128	160	400	4	256	8	64	128	200	< 4	32
C. G.	< 4	< 4	< 4	< 4	< 4	16	16	128	32	64	< 4	< 4
C. G.	128	256	256	800	128	1280	32	64	< 32	< 16	< 32	64
C. R.	32	–	128	–	< 4	–	< 4	–	4	–	< 4	–
E. W.	< 4	16	256	1280	< 4	1024	128	512	< 8	16	< 8	8
F. E.	< 4	4	320	256	32	< 4	512	< 8	64	< 8	10	< 8
G. J.	< 4	< 4	< 4	160	< 4	512	8	128	< 4	1024	< 4	128
G. E.	128	128	1024	320	< 4	16	–	512	–	1024	–	32
G. L.	13	32	32	64	13	128	32	> 2048	< 16	< 8	< 16	64
G. J. A.	< 4	< 4	< 4	< 4	< 4	< 4	< 16	32	< 16	< 4	< 16	< 4
H. L.	4	16	400	640	< 4	64	< 4	< 4	< 4	8	< 4	8
H. R.	64	128	256	640	512	1024	64	64	< 4	16	16	32
K. F.	512	> 2048	512	> 2048	> 2048	> 2048	1024	256	32	128	512	800
K. L.	< 4	< 4	< 4	< 4	< 4	> 2048	< 8	256	400	512	< 8	128
K. J.	32	< 4	32	< 4	320	4	–	32	–	128	–	640
K. S.	4	–	512	–	5	–	16	–	3	–	< 4	–
L. L.	5	32	32	160	64	200	128	640	16	128	16	40
M. C.	8	16	512	512	< 4	< 4	40	512	< 16	4	16	128
S. A.	20	40	13	32	128	256	16	512	< 8	8	< 8	128
S. D.	128	256	160	256	256	256	< 16	256	< 16	< 32	32	256
Z. K.	< 4	16	8	8	80	> 2048	< 16	1024	128	128	< 16	64
Z. L.	256	256	512	1280	< 4	64	640	> 2048	< 16	128	128	1024
Z. M.	< 4	8	< 4	128	< 10	1024	256	> 2048	< 4	< 8	< 4	> 2048

TABLE IV. PERCENTAGE INCREASES IN TITER GREATER THAN TWOFOLD AFTER INOCULATION

GROUP	TYPE 1	TYPE 2	TYPE 3	TYPE 4	TYPE 5	TYPE 7
A. One 0.25 c.c. subcutaneous inoculation followed by one 0.5 c.c. in 2 weeks	29	29	59	82	46	64
B. One 1.0 c.c. subcutaneous inoculation	13	26	57	7	7	54
C. One 0.1 c.c. intradermal inoculation	61	52	74	76	48	76

Timm,[5] using the same vaccine lots as were used in our study, obtained, in adults, a larger percentage showing greater than twofold increase in titer. The reason for higher titers in adults is not known. It cannot be inferred that allergic children are poor antibody producers. Possibly the answer lies in the fact that we used the vaccine six months later than Timm did, and there may have been a significant loss of potency.

Huebner and associates[6] produced titers greater than 1 to 8 in thirty-five of forty-five volunteers injected with type 3 vaccine, which indicated that significant antibody titers can be produced with fresh, potent vaccine in sufficiently high dosage.

Jordan and colleagues[7] reported a study of the age of acquisition of adenovirus antibodies. At the age of 5 years about 60 per cent of the sixty-three children reported had antibodies for type 2 adenovirus, 48 per cent for type 1, 27 per cent for type 6, 17 per cent for type 3, and 10 per cent for types 7 and 5. They concluded from their data, that clinical infection with types 3, 4, 5, and 7 adenovirus was uncommon before 5 years of age.

The subjects in our study were in the 7- to 12-year age group, and their prevaccination titers were 1 to 8 or greater in 46 per cent for type 1, 68 per cent for type 2, 44 per cent for type 3, 76 per cent for type 4, 60 per cent for type 5, and 48 per cent for type 7. Our results indicated that by the age of 7 to 12 years more than one-half of the tested children had acquired significant antibody titers and presumably had been clinically infected with the various adenoviruses. These figures tend to confirm the prevalence of infection due to the adenoviruses. We did no studies on morbidity among our children, however, and cannot associate acquisition of antibodies with clinical illness. We are not even certain that demonstrable titers are necessary for protection or that high antibody titer necessarily affords protection. If there is no definite correlation between antibody titer and degree of immunity, some other means of evaluating clinical protection must be evolved.

We produced higher titers with a small intradermal dose than with a larger subcutaneous dose. It has been known that typhoid vaccine injected intradermally will produce a good antibody response, but it has not been universally accepted that such is the case with virus vaccines. Should further studies indicate that the intradermal route is the best method of producing high antibody titers, this will be of considerable practical importance because of the smaller quantity of vaccine required, the reduced cost of the vaccine, and the reduced incidence of reactions to the vaccine.

SUMMARY AND CONCLUSIONS

Sixty-three children, 7 to 12 years of age, were injected with polyvalent adenovirus vaccine. Pre- and postvaccination titers are recorded. Unexpectedly high preinjection titers were found.

We draw no conclusions from the data presented but wish to point out that we produced higher titers via the intradermal route than via the subcutaneous route.

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GROWTH SUPPRESSION IN ASTHMATIC CHILDREN RECEIVING PROLONGED THERAPY WITH PREDNISONE AND METHYLPREDNISOLONE

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THERE is considerable reported evidence indicating that adrenal cortical hormones of the cortisone type can suppress linear growth. Children with Cushing's syndrome due to hypersecretion of these hormones grow more slowly than normal children.[1] Experimental animals treated with either cortisone or ACTH grow at a reduced rate.[2-4] In children treated for prolonged periods with sufficient cortisone, there is evidence of growth retardation which may be reversed by discontinuing therapy or by reducing dosage below a critical growth-suppressive level.[5-6]

There are also recorded observations indicating that chronic bronchial asthma may be associated with growth retardation.[7-8]

This report is concerned with the growth of children with severe, persistent, chronic bronchial asthma who receive prolonged corticosteroid therapy.

Since January, 1955, in the Pediatric Allergy Clinic of The Johns Hopkins Hospital, observations have been made of the growth of thirteen children with intractable perennial asthma who have received prednisone, prednisolone, or methylprednisolone† more or less continuously for six months or longer. Table I shows the age, race, and sex of each patient, the duration of observation before, during, and after corticosteroid therapy, and the average dosage employed. Steroid dosage is expressed in milligrams per square meter of body surface per day in order to facilitate comparison of dosages employed in patients of different size.[5]

Since March, 1957, a similar study has been made of the growth of fifty-two children with perennial bronchial asthma who have received no corticosteroid therapy.

Height and weight were measured with the patients in the standing position and recorded on Stuart anthropometric charts‡ (Fig. 1).[5] With these charts,

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†Prednisolone was supplied as Delta-Cortef and methylprednisolone was supplied as Medrol by The Upjohn Company, Kalamazoo, Michigan.

‡Stuart anthropometric charts were supplied by Mead Johnson & Company, Evansville, Indiana.

observed and normal increase in weight and height during any observation period could be determined and compared. The linear growth rate in per cent of normal during a given period of observation was calculated by dividing the observed increase in height by the normal increase in height and multiplying by 100. The weight gain in per cent of normal during a given period of observation was calculated by dividing the observed increase in weight by the normal increase in weight and multiplying by 100.

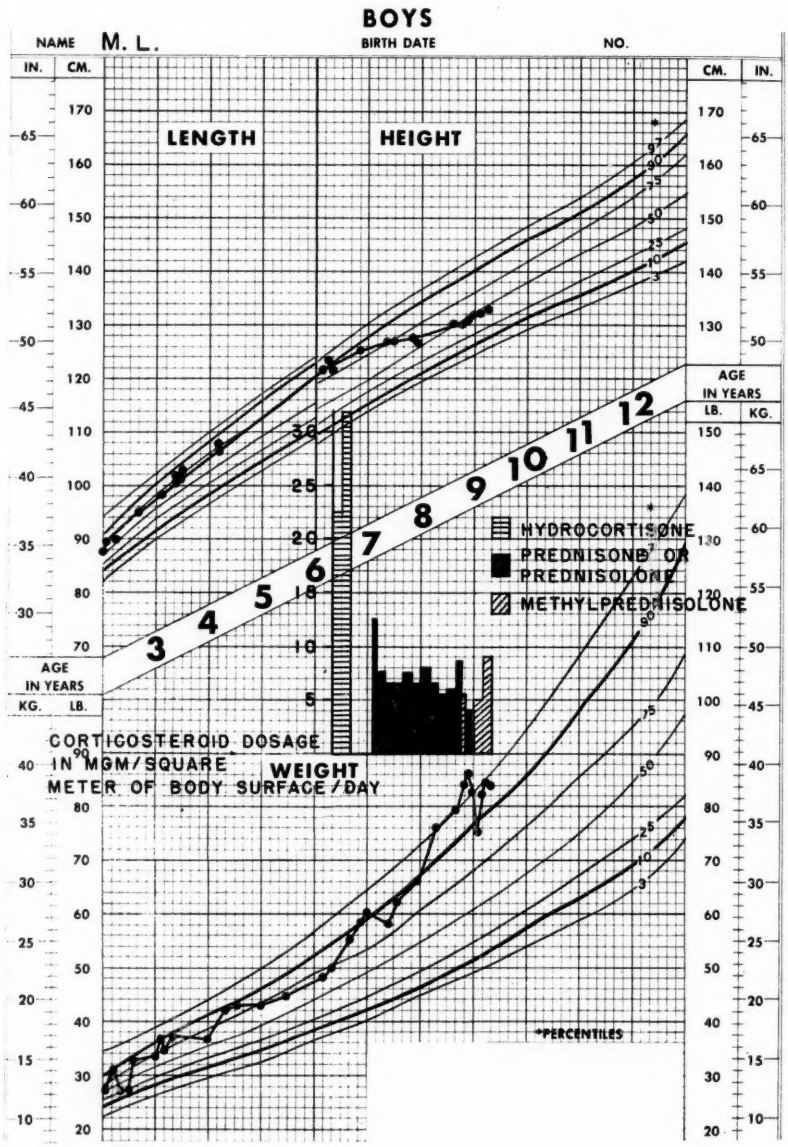

Fig. 1.—Patient M. L. Height and weight measurements and average daily corticosteroid dosage have been plotted on a Stuart anthropometric chart.

RESULTS

Observations of Linear Growth and Weight Gain in Thirteen Asthmatic Children Receiving Prolonged Corticosteroid Therapy.—The data on linear growth are shown in Table I and Fig. 2. Before onset of corticosteroid therapy, linear growth rate was essentially normal. During therapy with prednisone or methylprednisolone growth retardation was evident when the average dosage for a six-month or longer period of observation exceeded 5.0 mg. per square meter of body surface per day, and growth was normal when the average dosage was less. The degree of growth retardation was roughly proportional to steroid dosage and appeared similar with either prednisone or methylprednisolone. After cessation of corticosteroid therapy, linear growth rate was accelerated.

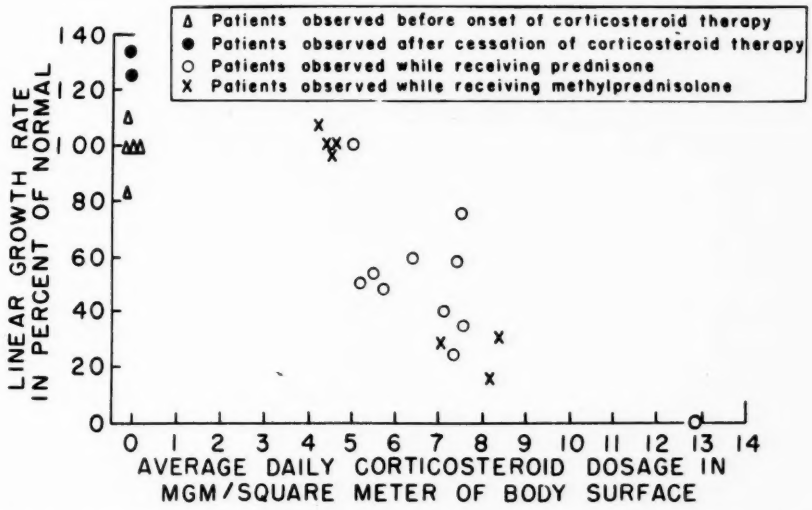
Δ Patients observed before onset of corticosteroid therapy
● Patients observed after cessation of corticosteroid therapy
○ Patients observed while receiving prednisone
X Patients observed while receiving methylprednisolone
LINEAR GROWTH RATE IN PERCENT OF NORMAL
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
AVERAGE DAILY CORTICOSTEROID DOSAGE IN MGM/SQUARE METER OF BODY SURFACE

Fig. 2.—Relationship between average daily corticosteroid dosage and linear growth rate in the thirteen patients presented in Table I. Each plotted point represents data derived from at least six consecutive months of observation.

The data on weight gain are shown in Table I and Fig. 3. Before onset of corticosteroid therapy, weight gain was more often subnormal than normal. During therapy with prednisone or methylprednisolone, weight gain was usually normal or accelerated, even when linear growth was retarded. After cessation of corticosteroid therapy, weight gain tended to be normal.

Those children exhibiting growth retardation while on steroid therapy were well nourished and even obese. At least five developed moon facies. Roentgenograms revealed normal bone age and structure in nine, moderate osteoporosis in two, and moderate retardation of bone age consistent with height age in two. Determinations of serum protein-bound iodine were normal in the eleven patients tested. No other untoward side effects of therapy were noted. Control of asthma was adequate in all.

TABLE I. SUMMARY OF DATA ON THIRTEEN PATIENTS WITH CHRONIC ASTHMA WHO RECEIVED CORTICOSTEROID THERAPY FOR SIX MONTHS OR LONGER

PATIENT	RACE	SEX	AGE AT ONSET OF ASTHMA (YEARS)	DURATION OF OBSERVATION PERIOD (MONTHS)	AGE DURING OBSERVATION PERIOD (YEARS)	CORTICOSTEROID GIVEN DURING OBSERVATION PERIOD	AVERAGE DAILY CORTICOSTEROID DOSE IN MG. PER SQ. M. OF BODY SURFACE PER DAY DURING OBSERVATION PERIOD	LINEAR GROWTH RATE IN PER CENT OF NORMAL	WEIGHT GAIN IN PER CENT OF NORMAL
J. M.	N	M	1.3	2	1.5- 1.7	None	0	100	100
				2	1.8- 2.0	Hydrocortisone	36	0	ND
				22	2.0- 3.8	Prednisone	12.9	0	45
				1	3.8- 3.9	Prednisone	8.5	75	100
				3	3.9- 4.2	Methylprednisolone	8.5		
H. W.	N	M	0.5	20	2.5- 4.2	Prednisone	7.5	75	67
D. A.	W	F	1.4	12	4.0- 5.0	Prednisone	7.4	57	100
				6	5.0- 5.5	Methylprednisolone	4.4	100	−133
S. S.	W	F	3.2	13	3.8- 4.9	None	0	113	60
				11	4.9- 5.8	Prednisone	6.4	60	200
				8	5.8- 6.0	None	0	125	100
L. G.	N	M	1.3	11	4.2- 5.1	None	0	83	50
				6	5.1- 5.6	Methylprednisolone	8.2	17	250
				5	5.6- 6.1	None	0	120	100
D. L.	N	M	2.0	24	3.6- 5.9	None	0	100	129
				9	5.9- 6.5	Prednisone	7.6	33	100
				7	6.5- 7.1	Methylprednisolone	7.1	29	100
				4	7.1- 7.5	None	0	200	135

C. M.	W	F	1.2	3	5.8- 6.1	Hydrocortisone	31.0	69	50
				23	6.1- 8.0	Prednisone	7.3	69	50
				7	8.0- 8.6	Methylprednisolone	4.6	100	150
M. L.	N	M	0.8	52	2.0- 6.3	None	0	100	100
				4	6.3- 6.7	Hydrocortisone	27.0	ND*	400
				5	6.7- 7.1	None	0	ND*	100
				22	7.1- 8.8	Prednisone	7.1	40	170
				1	8.8- 8.9	Methylprednisolone	5.5	100	–400
				2	8.9- 9.0	Prednisolone	4.0		
				4	9.0- 9.3	Methylprednisolone	7.6	50	300
S. J.	N	M	6.8	8	7.5- 8.2	Methylprednisolone	8.4	30	117
T. D.	W	M	4.0	9	8.3- 9.0	Prednisone	5.7	50	110
				4	9.0- 9.3	Methylprednisolone	4.5	25	0
				6	9.3- 9.8	None	0	133	133
E. R.	W	F	1.5	17	9.3-11.0	Prednisone	5	100	386
R. G.	N	M	9.0	14	9.6-10.8	Prednisone	5.1	50	72
				6	10.8-11.2	Methylprednisolone	4.4	100	143
				3	11.2-11.5	None	0	260	100
D. G.	N	M	7.8	25	7.8- 9.9	None	0	100	40
				23	9.9-11.8	Prednisone	5.4	55	189
				6	11.8-12.4	Methylprednisolone	4.25	110	150
				4	12.4-12.8	None	0	160	33

*ND = Not determined.

Observations of the Linear Growth and Weight Gain of Fifty-Two Asthmatic Children Receiving no Corticosteroid Therapy.—Linear growth rate was essentially normal during the ten-month or longer period of observation in all patients studied. It was 80 to 89 per cent of normal in four patients, 90 to 109 per cent of normal in forty-two, 110 to 129 per cent of normal in four, and 130 to 135 per cent of normal in two.

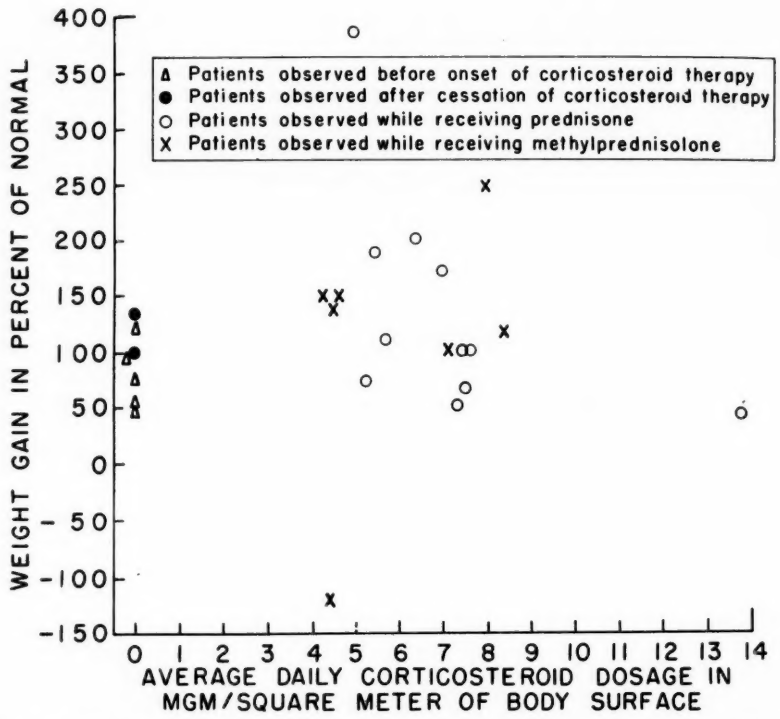

Fig. 3.—Relationship between average daily corticosteroid dosage and weight gain in the thirteen patients presented in Table I. Each plotted point represents data derived from at least six consecutive months of observation.

Weight gain during the same period of observation was more variable, being 30 to 49 per cent of normal in two patients, 50 to 69 per cent of normal in seven, 70 to 89 per cent of normal in four, 90 to 109 per cent of normal in twenty-one, 110 to 129 per cent of normal in seven, 130 to 149 per cent of normal in three, 150 to 169 per cent of normal in six, and 170 to 189 per cent of normal in two.

CASE REPORTS

Case 1, Patient M. L.—Severe perennial asthma began at the age of 9 months and was intractable despite extensive therapy, including avoidance of allergens, desensitization, psychotherapy, bronchodilators, expectorants, and antibiotics. However, linear growth (Fig. 1) proceeded along the seventy-fifth percentile at 100 per cent of the normal rate until corticosteroid therapy was begun. Between the ages of 7 years 1 month and 9 years 5 months, the patient received prednisone, prednisolone, and methylprednisolone, alone or in combination, in an average dosage of 7 mg. per square meter of body surface per day (7.4 mg. per day). Concomitantly, despite improvement of asthma and accelerated weight gain, linear growth

occurred at 47 per cent of the previous normal rate. The slowing was sufficiently subtle and variations in height measurements were sufficiently large to necessitate prolonged observation for conclusive demonstration of retardation of growth. After two years of steroid therapy, the boy was obese and moon-faced.

Case 2, Patient J. M.—Severe perennial asthma began at the age of 1 year 7 months and was intractable despite extensive therapy, including avoidance of allergens, desensitization, bronchodilators, expectorants, and antibiotics. However, linear growth (Fig. 4) proceeded along the ninetieth percentile at 100 per cent of the normal rate until corticosteroid therapy was begun. Between the ages of 2 years and 3 years 10 months, the patient received

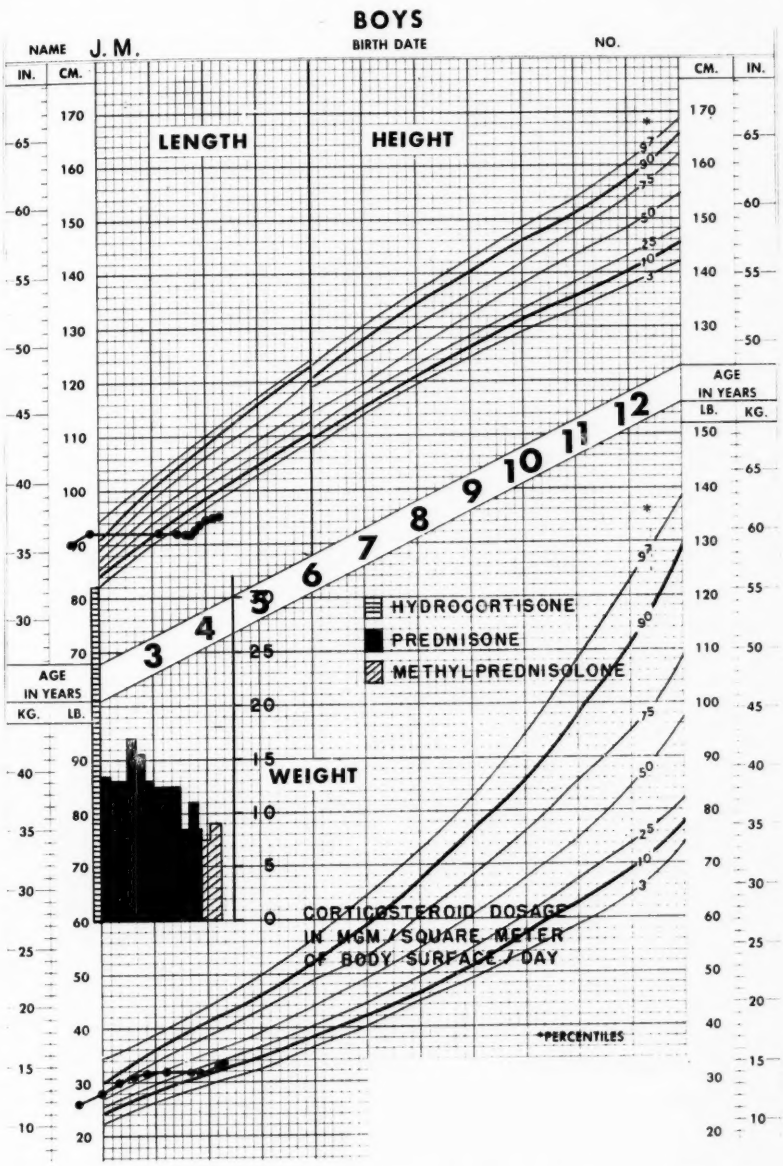

Fig. 4.—Patient J. M. Height and weight measurements and average daily corticosteroid dosage have been plotted on a Stuart anthropometric chart.

prednisone in an average dosage of 12.9 mg. per square meter of body surface per day (7.5 mg. per day). Concomitantly, despite improvement of asthma, growth ceased entirely and he became a moon-faced, symmetrical dwarf (Fig. 5). At the age of 3 years 10 months, reduction in steroid dosage was followed by resumption of growth.

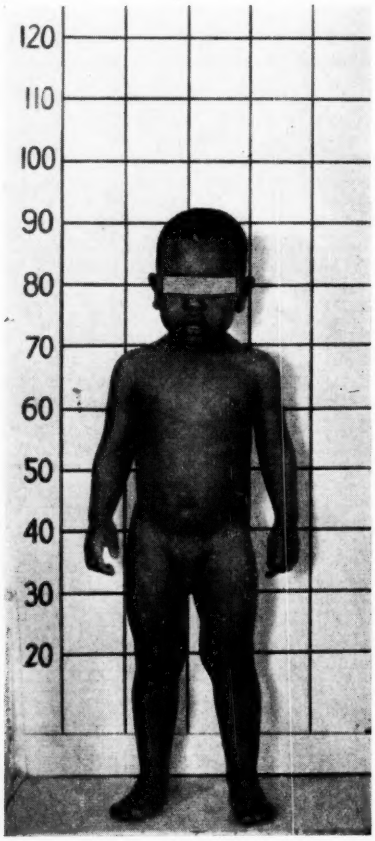

Fig. 5.—Photograph of Patient J. M. at the age of 3 years 8 months after twenty-two consecutive months of corticosteroid therapy. Symmetrical dwarfing and moon facies are evident.

Case 3, Patient D. G.—Severe perennial asthma began at the age of 7 years 10 months and was intractable despite extensive therapy, including avoidance of allergens, desensitization, bronchodilators, and antibiotics. However, linear growth (Fig. 6) proceeded along the twenty-fifth percentile at 100 per cent of the normal rate until corticosteroid therapy was begun. Between the ages of 9 years 11 months and 11 years 10 months, the patient received prednisone in an average dosage of 5.4 mg. per square meter of body surface per day (5.1 mg. per day). Concomitantly, despite improvement of asthma and accelerated weight gain, growth proceeded at only 55 per cent of the previous normal rate. Moon facies was never definitely evident. Between the age of 11 years 10 months and 12 years 4 months, methylprednisolone was given in an average dosage of 4.25 mg. per square meter of body surface per day (4.8 mg. per day), and the linear growth rate increased to 110 per cent of the normal level observed prior to any corticosteroid therapy. Between the ages of 12 years 4 months and 12 years 9 months, steroid therapy was discontinued and the linear growth rate rose to 160 per cent of normal.

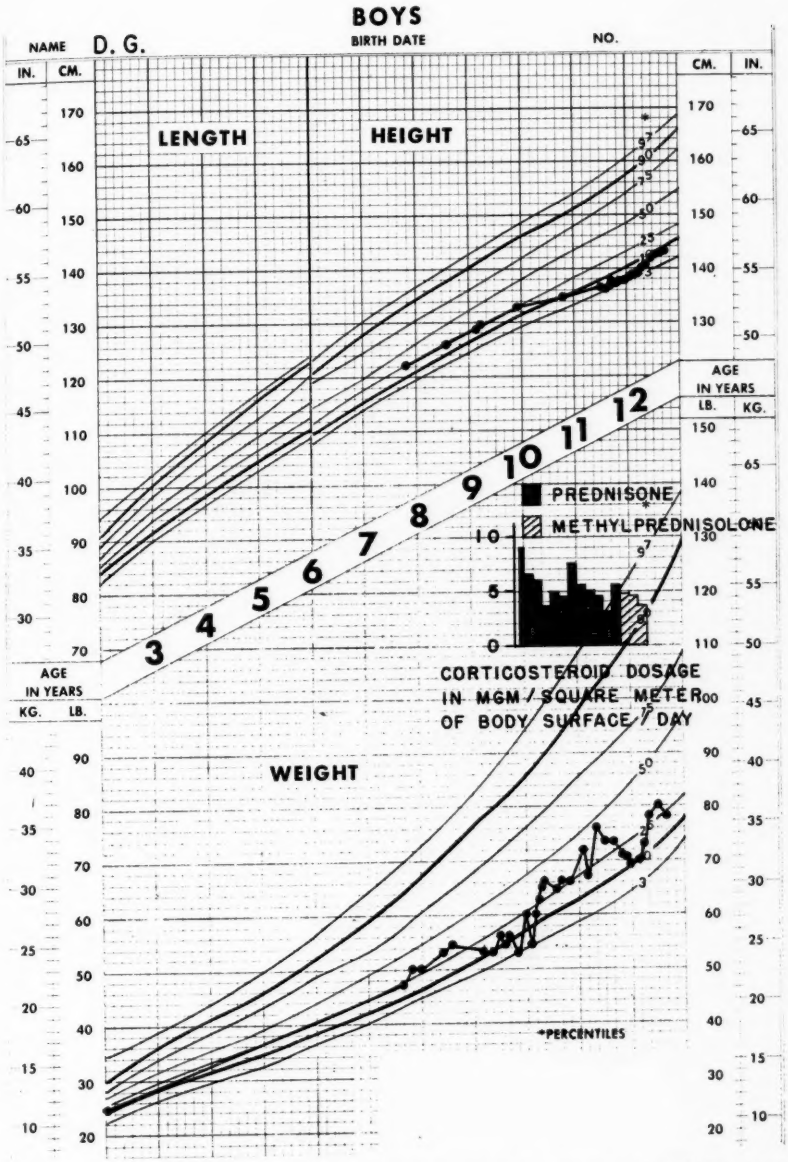

Fig. 6.—Patient D. G. Height and weight measurements and average daily corticosteroid dosage have been plotted on a Stuart anthropometric chart.

DISCUSSION

The linear growth retardation observed in the asthmatic children receiving prednisone and methylprednisolone can be logically attributed to steroid therapy. It first appeared at the time of onset of such therapy. The degree of inhibition was roughly proportional to the average dosage employed. Cessation of therapy or reduction of average dosage below the critical growth-suppressive level was followed by the appearance of a normal or accelerated growth rate.

Chronic asthma and inanition have been reported to be associated with growth suppression,[7, 8] but there is little evidence that these factors were responsible for growth suppression in the corticosteroid-treated children described here. In general, before corticosteroid therapy asthma was severe and growth was normal; whereas during therapy asthma and nutrition were improved and growth was retarded as long as sufficient steroid was given. Observations of the growth of fifty-two patients with asthma sufficiently mild to require no steroid therapy indicated essentially normal growth.

Evidence derived from studies of experimental animals has indicated that adrenal cortical hormones suppress linear growth through inhibition of epiphyseal cartilage cell proliferation and that this effect is antagonistic to the skeletal effect of pituitary growth hormone.[9] A direct inhibitory effect on growth of fibroblasts in tissue culture has also been shown.[10] This growth-suppressive effect "may be attributable to a protein catabolic effect, to inhibition of protein anabolism, or merely to excessive withdrawal of amino acids from the metabolic pool for the purpose of gluconeogenesis."[11] Moon facies has been observed frequently in children with growth suppression associated with corticosteroid therapy.[5]

The critical growth-suppressive dosage of prednisone in the patients reported here was 5.1 mg. per square meter of body surface per day; the critical growth-suppressive dosage of cortisone in the patients reported by Blodgett and associates[5] was 45 mg. per square meter of body surface per day. These two figures were derived in different groups of patients by different observers, and it is therefore hazardous to compare them. Since prednisone is only four to five times more potent than cortisone as a therapeutic agent, however, any indication that it may be nine times more potent than cortisone as a growth-suppressive agent should be confirmed or disproved by further study.

It seems essential to follow carefully the growth of children receiving prolonged steroid therapy. Dosage should be reduced below growth-suppressing levels sufficiently often to permit recovery of any growth lost. This must be done before the age when further skeletal growth is precluded by closure of skeletal epiphyses.[5]

SUMMARY

Growth suppression was noted in twelve of thirteen asthmatic children receiving sufficient prednisone or methylprednisolone for six months or longer. Evidence indicating that this growth retardation could be logically attributed to the corticosteroid therapy is presented.

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SEROLOGIC STUDIES ON FAVISM

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FAVISM is a disease which is characterized by an acute hemolytic anemia with hematuria and jaundice. It is produced by the ingestion of the broad bean *(Vicia fava)* or by inhalation of its pollen when the plant is in blossom. Although the disease has been known for centuries, it was first reported in this country in 1933.[1-2] Since then more cases have been reported in the United States literature,[3-14] and in 1941 the first excellent review in our literature about favism was made by Louisada.[15] Also, cases of asthma and hay fever due to inhalation of the fava bean pollen have been reported.[2]

Because favism is predominantly found in the Mediterranean countries, chiefly Italy, the pathogenesis of the disease was quite intensively studied by workers of these countries.[11, 15-26] However, the etiology of the disease has not yet been completely elucidated. Is there a toxin present within the fava bean which affects the red blood cells of populations of oriental origin, no matter in which part of the world they live? Or is there an antigen-antibody reaction which affects the red blood cells or the whole reticuloendothelial system as shock organ?

Reviewing immunologic studies in connection with favism, Marcolongo and associates[21] favored the theory of autoimmunization and autosensitization similar to those of the majority of other forms of acquired hemolytic anemias. Their interpretation is generally based on the presence of atypical antibodies against the red blood cells in the patient's sera.

In this serologic study, a hemagglutinating antibody against fava bean was found in immune antifava rabbit sera, as well as in the serum of a patient with favism, which was taken in the *acute stage* of the disease prior to any therapy such as blood transfusion.

As the first step of this investigation, immune antifava rabbit serum was tested.

MATERIAL AND METHODS

Sera.—Two rabbits were immunized with fava bean extract mixed with an equal volume of Freund's adjuvant. One rabbit was given four subcutaneous injections in the back at different sites at the same time.† This rabbit was

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†This animal was kindly immunized by Dr. Jacob Pruzansky at the Allergy Laboratory of the Northwestern University School of Medicine, Chicago, Illinois.

bled after 5 weeks and the serum was stored (R anti F_1). The other rabbit was immunized with the similar extract mixed with Freund's adjuvant; this animal received an intradermal injection in each of the four footpads. A trial bleeding three weeks after immunization demonstrated antibodies against fava by the hemagglutination method; the animal was then sacrificed by cardiac puncture on the following day, and the serum was prepared (R anti F_2).

Because of the rarity of the disease in the United States, we were able to obtain only two sera from local patients who had favism several years ago (sera "C" and "D"). One of them had favism caused by inhalation of the fava bean pollen. Four other sera (sera "A," "B," "L," and "M") were kindly supplied by the Children's Department of the Hadassah Hospital in Tel Aviv, Israel.* One of those sera (serum "L") was taken from a patient in an acute state of the disease before transfusion was given. Another serum from the same source was from the mother of this patient, who did not have favism (serum "M"). The sera from two other patients, taken a few days after the onset of the disease and after transfusion was given, were also available (sera "A" and "B").

Normal rabbit serum 1:100 diluted in buffered saline pH 7.2 was used as diluent for the antisera, and normal rabbit serum in different titrations served as a control.

Normal human serum was pooled, and individual sera were used for control. All sera were inactivated at 56° C. for thirty minutes.

Antigens.—Ten per cent saline extracts of dried, defatted fava, lima, navy, and kidney beans were passed through a Seitz filter and cultures were taken for sterility.

After preliminary experiments with various dilutions of these 10 per cent extracts, it was found that the most suitable dilutions for treating the red blood cells were fava bean 1:10, lima bean 1:10, kidney bean 1:40, and navy bean also 1:40. This will be described in greater detail in the discussion.

Red Blood Cells.—Human red blood cells of group "O," Rh negative, were used for the experiments.

Buffered Saline.—The following solutions were used:

(1) pH 7.2 buffered saline, 0.15 M. solution
8.15 Gm. Na_2HPO_4
2.45 Gm. KH_2PO_4
4.5 Gm. NaCl
1,000 c.c. distilled H_2O

(2) pH 6.4 buffered saline, 0.15 M. solution
3.44 Gm. Na_2HPO_4
6.92 Gm. KH_2PO_4
4.5 Gm. NaCl
1,000 c.c. distilled H_2O

*We are thankful to Dr. Ben Z. Werbin, Head of the Pediatrics Department "B" at the Municipal Hospital, "Hadassah," in Tel Aviv, Israel, for supplying the patients' sera which made this study possible.

The buffered salines were checked by pH meter.

Normal Saline.—The normal saline used was 0.85 per cent of NaCl solution.

Tannic Acid.—A 1:200 solution of tannic acid crystal ($C_{14}H_{10}O_9$) (not fluffy) in H_2O was kept in stock for as long as ten days and diluted 1:20,000 in normal saline for the experiment.

METHOD OF HEMAGGLUTINATION

Since Neter's[27] excellent review of hemagglutination in 1956, more sensitive methods have been developed. The technique employed in the following experiments were basically similar to those described by Boyden[28] and modified by Stavitsky.[29] However, we carried out those studies with additional changes in methods so aptly worked out and developed by Witebsky and Rose[30] and others.[31, 32]

The details of these modifications are as follows:

(1) 0.5 ml. of the antisera was prepared in serial dilutions in normal rabbit serum 1:100, as were controls of normal human sera and normal rabbit sera.

(2) Thrice washed in 7.2 buffered saline, citrated human group O, Rh-negative red blood cells were resuspended to 2.5 per cent in pH 7.2 buffered saline and treated with equal parts of tannic acid 1:20,000 for ten minutes at 37° C. After centrifugation for five minutes at 1,200 r.p.m., the red blood cells were washed in the same volume of pH 7.2 buffered saline, and the tanned red blood cells were then resuspended in normal saline to 2.5 per cent. The cells were "sensitized" by adding one part of red blood cells to four parts of pH 6.4 buffered saline and one part of antigen. This mixture was then incubated for fifteen minutes at room temperature and centrifuged. After one washing with two volumes of 1:100 normal rabbit serum, the tanned sensitized red blood cells were made up as a 2.5 per cent suspension in normal rabbit serum 1:100, and 0.05 c.c. was added to serial dilutions of antisera.

(3) Controls consisted of normal rabbit serum, normal human serum with tanned sensitized red blood cells, and also test sera with tanned but unsensitized red blood cells.

(4) The tubes were shaken, and the hemagglutination patterns were recorded after two hours at room temperature and again after they had remained in the icebox overnight.

(5) The hemagglutination patterns remain stable, without lysis, for as long as twenty-four hours if kept at room temperature and for three to four days if kept in the icebox. There was no apparent elution of antigen.

METHOD OF INHIBITION TEST

After preliminary experiments, the following technique for the inhibition test was adopted:

Serial dilutions of each of the various antigens (fava, lima, navy, and kidney bean) diluted in normal rabbit serum 1:100 were set up in a volume of 0.1 c.c. per tube. A constant amount of 0.5 c.c. of a 1:20,000 dilution of immune antifava rabbit serum, or a dilution of 1:150 of the favism patient's serum diluted in normal rabbit serum 1:100, was added to each tube as well as to the control tubes. Tubes were shaken and allowed to stand for two hours at room temperature. Tanned human red blood cells were then sensitized, as in the hemagglutination method, with the fava bean extract in the usual manner, and 0.05 c.c. was added to each tube. The patterns were read after two hours at room temperature and again after they had been in the icebox overnight.

As in the hemagglutination test, the patterns remain stable for twenty-four hours at room temperature and for three to four days if kept in the icebox.

RESULTS

Hemagglutination Tests.—Table I demonstrates that both antifava rabbit sera contained hemagglutinins in high titers (1:64,000 and 1:128,000), but no such antibody was present in the normal rabbit serum and normal human serum controls.

TABLE I. HEMAGGLUTINATION OF TANNED RED BLOOD CELLS TREATED WITH 10 PER CENT FAVA BEAN EXTRACT (DILUTED 1:10) AFTER ADDITION TO ANTIFAVA RABBIT SERA

DILUTIONS OF ANTISERA IN NORMAL RABBIT SERUM 1:100	ANTIFAVA RABBIT SERUM		NORMAL RABBIT SERUM	NORMAL HUMAN SERUM
	1	2		
(1) 1:10	++++	++++	–	–
(2) 1:100	++++	++++	–	–
(3) 1:1,000	++++	++++	–	–
(4) 1:2,000	++++	+++	–	–
(5) 1:4,000	+++	++	–	–
(6) 1:8,000	++	+++	–	–
(7) 1:16,000	++	+++	–	–
(8) 1:32,000	+	+++	–	–
(9) 1:64,000	+	+++	–	–
(10) 1:128,000	±	+	–	–
(11) 1:256,000	–	–	–	–
(12) 1:512,000	–	–	–	–
(13) Antiserum control	–	–	–	–

++++ = Strong folding of edges in bottom of test tube.
+++ = Moderate folding of edges in bottom of test tube.
++ = Slight folding of edges in bottom of test tube.
+ = Diffuse bottom of test tube.
± = Very slight circle in bottom of test tube.
– = Circle in bottom of test tube.
Antiserum control = 1:10 dilution of antiserum plus tanned nonsensitized red blood cells.

To exclude the possibility of nonspecific hemagglutination of those immune rabbit sera to fava extract, the same sera were also tested for hemagglutination by sensitizing red blood cells with other bean extracts, such as lima bean, kidney bean, and navy bean. As a positive control they were sensitized, also with fava bean extract.

TABLE II. HEMAGGLUTINATION OF TANNED RED BLOOD CELLS TREATED WITH EXTRACTS OF FOUR BEAN ANTIGENS

DILUTIONS OF ANTIFAVA RABBIT SERUM NO. 2	RED BLOOD CELLS TREATED WITH			
	FAVA BEAN 1:10	NAVY BEAN 1:40	KIDNEY BEAN 1:40	LIMA BEAN 1:10
(1) 1:10	++++	++	–	+
(2) 1:100	+++	++	–	+
(3) 1:1,000	+++	–	–	–
(4) 1:2,000	+++	–	–	–
(5) 1:4,000	+++	–	–	–
(6) 1:8,000	+++	–	–	–
(7) 1:16,000	+++	–	–	–
(8) 1:32,060	+++	–	–	–
(9) 1:64,000	+++	–	–	–
(10) 1:128,000	++	–	–	–
(11) 1:256,000	–	–	–	–
(12) Normal rabbit serum control	–	–	–	–
(13) Antiserum control	–	–	–	–

Table II illustrates specific hemagglutination of treated red blood cells caused by the above immune antifava rabbit sera when sensitized with fava bean extract but no reaction (or only a very questionable reaction) when sensitized with other bean extracts.

Inhibition Tests.—In order to test the specificity of the hemagglutination reactions, inhibition tests were also carried out.

As can be seen from Table III, fava bean extract in a dilution of 1:10,935 inhibited hemagglutination of red blood cells sensitized with fava bean extract by immune antifava rabbit serum 1:20,000, but no inhibition could be demonstrated with the other bean extract antigens.

TABLE III. INHIBITION OF HEMAGGLUTINATION OF ANTIFAVA RABBIT SERUM NO. 2 (1:20,000 DILUTION) BY ADDITION TO VARYING DILUTIONS OF FOUR BEAN EXTRACTS PRIOR TO THE FURTHER ADDITION OF RED BLOOD CELLS TREATED WITH FAVA BEAN EXTRACT 1:10*

DILUTIONS OF ANTIGENS IN NORMAL RABBIT SERUM 1:100 0.1/TUBE	FAVA BEAN	LIMA BEAN	KIDNEY BEAN	NAVY BEAN
(1) 1:5	–	++	+	+
(2) 1:15	–	++	++	+
(3) 1:45	–	++	++	++
(4) 1:135	–	++	++	++
(5) 1:405	–	++	++	++
(6) 1:1,215	–	++	++	++
(7) 1:3,645	–	++	++	++
(8) 1:10,935	–	++	+	+
(9) 1:32,805	+	+	+	+
(10) 1:98,415	++	+	+	+
(11) Normal rabbit serum	+	+	+	+
(12) 1:5 plus tanned untreated red blood cells	–	–	–	–

*See text for method of inhibition test.

STUDIES WITH FAVISM PATIENTS' SERA

Since we were able to demonstrate specific circulating antibodies in a high titer in immune antifava rabbit sera, the question as to whether such an antibody is also present in sera of patients with favism arose.

TABLE IV. HEMAGGLUTINATION OF TANNED RED BLOOD CELLS TREATED WITH FAVA BEAN EXTRACT (10 PER CENT DILUTED 1:10)

DILUTIONS OF PATIENTS' SERA IN NORMAL RABBIT SERUM 1:100	PATIENT L.	PATIENT A.	PATIENT B.	PATIENT M.	PATIENT C.	PATIENT D.	POOLED NORMAL HUMAN SERUM
(1) 1:15	++++	–	–	–	–	–	–
(2) 1:30	+++	–	–	–	–	–	–
(3) 1:60	+++	–	–	–	–	–	–
(4) 1:120	++	–	–	–	–	–	–
(5) 1:240	++	–	–	–	–	–	–
(6) 1:480	+	–	–	–	–	–	–
(7) 1:960	–	–	–	–	–	–	–
(8) Normal rabbit serum control	–	–	–	–	–	–	–
(9) Patients' sera 1:15 plus tanned untreated red blood cells	–	–	–	–	–	–	–

Experiments similar to those with the immune antifava rabbit sera were carried out with the patients' sera. Table IV and V illustrate a positive reaction (titer 1:480 and 1:640) in the serum of Patient L. (serum taken in acute state of the disease, before transfusion was given). The other patients, whose sera were taken after transfusion, failed to show any positive hemagglutination. The sera of patients who had favism several years ago likewise showed no visible reaction. The control sera were negative.

Patient L.'s serum was tested for specificity with other bean extracts. Table V illustrates specific hemagglutination in a titer of 1:640 when tested with fava bean extract and no titer or only a very slight reaction (1:20) when red blood cells were sensitized with other bean extracts (navy and kidney beans 1:20, lima bean 0).

TABLE V. HEMAGGLUTINATION OF TANNED RED BLOOD CELLS TREATED WITH EXTRACTS OF FOUR BEAN ANTIGENS

DILUTIONS OF PATIENT L.'S SERUM IN NORMAL RABBIT SERUM 1:100	RED BLOOD CELLS TREATED WITH			
	FAVA BEAN 1:10	LIMA BEAN 1:10	KIDNEY BEAN 1:40	NAVY BEAN 1:30
(1) 1:20	++++	–	+	+
(2) 1:40	++++	–	–	–
(3) 1:80	+++	–	–	–
(4) 1:160	++	–	–	–
(5) 1:320	+	–	–	–
(6) 1:640	+	–	–	–
(7) 1:1,280	–	–	–	–
(8) Normal rabbit serum control	–	–	–	–
(9) Normal human serum control	–	–	–	–
(10) Serum L. 1:20 plus tanned untreated red blood cells	–	–	–	–

Table VI illustrates specific inhibition of fava bean extract in a dilution of 1:135 when in reaction with Patient L.'s serum diluted 1:150. No inhibition was demonstrated by lima and kidney bean, and very slight inhibition (1:15) was demonstrated by navy bean.

TABLE VI. INHIBITION OF HEMAGGLUTINATION OF PATIENT L.'S SERUM (1:150 DILUTION) BY ADDITION OF 0.5 C.C. PER TUBE TO VARYING DILUTIONS OF FOUR BEAN EXTRACTS PRIOR TO THE FURTHER ADDITION OF RED BLOOD CELLS TREATED WITH FAVA BEAN EXTRACT*

DILUTIONS OF ANTIGENS IN NORMAL RABBIT SERUM 1:100 0.1/TUBE	FAVA BEAN	LIMA BEAN	KIDNEY BEAN	NAVY BEAN
(1) 1:5	–	++	+	–
(2) 1:15	–	+++	+++	–
(3) 1:45	–	+++	+++	+
(4) 1:135	–	+++	+++	+
(5) 1:405	+	+++	+++	++
(6) 1:1,215	++	+++	+++	++
(7) Normal rabbit serum control	++	+++	+++	++
(8) 1:5 plus tanned, untreated red blood cells	–	–	–	–

*See text for method of inhibition test.

DISCUSSION

The pathogenesis of favism is not definitely known. A toxic mechanism is suggested by the presence of plant agglutinins in the bean families, including fava bean.[18-20, 30] It is interesting to note that in our studies we found that fava bean extract in dilutions greater than 1:5 did not produce lysis of the tannic-acid-treated red blood cells in the test tube. However, navy bean and kidney bean extracts, which do not produce hemolytic disease in vivo, did cause lysis of these cells in vitro in dilutions of 1:20 and 1:30, respectively.

Others compare favism to the drug-induced hemolytic anemias (due to sulfonamide drugs, primaquine, or naphthalene) and show comparable evidence of metabolic changes, such as glutathione deficiency in the red blood cells of those patients.[17, 33-38] Although those authors do not exclude a hereditary and individual hypersensitivity factor in the disease, they ignored a true immunologic hypothesis.

Other authors conceived the allergic theory.[11-16, 21-26] Glaser[39] defined favism as "a disease due to allergy to the fava bean." Preti[40] was the first to use the term "anaphylactic syndrome" in connection with favism. According to Winthrobe,[41] incomplete red blood cell antibodies are present in the patient's sera. In his review of the immunology of hemolytic anemias, Milgrom[42] stated that autoimmunization occurs in hypersensitive persons. Besides in vitro and animal experiments,[22-25] it is established that direct skin tests, as well as Prausnitz-Küstner tests, are positive in patients after recovery but are absent in the acute stage of the disease (desensitization phenomenon?). It is also well known that favism does not occur on the first ingestion of the bean or on first exposure to its pollen.[15] The patient has to be first sensitized to become sick. Eosinophilia is always present during an attack. There are also observations that when prolonged rain produces a low-pollen season, the frequency of favism is diminished.[16]

Although a hemagglutinating antibody was found in the serum of an untreated patient with favism, this does not preclude allergy or immunology as the pathogenic factor in favism. Other factors mentioned above must be

considered as etiological agents. To our knowledge, however, this is the first report in which antibodies were demonstrated by means of hemagglutination to the fava bean in vitro in an untreated patient suffering from favism.

It is hoped that this finding will stimulate other workers in this field, where the disease is more prevalent, to carry on further studies along these lines.

SUMMARY AND CONCLUSIONS

1. A substance which was present in the serum of a patient with acute untreated favism and in sera of rabbits immunized with fava bean extract had the ability to cause agglutination of human group "O," Rh-negative red blood cells previously treated with tannic acid and exposed to fava bean extract.

2. The reaction was specific, and hemagglutination of tanned red blood cells treated with extracts from other types of beans was minimal or did not occur. The specificity was also confirmed by the specific inhibition reaction.

3. Patients with favism who had been treated by blood transfusions and recovered failed to show this type of antibody.

4. Although these findings tend to support the immunologic etiology of favism, such a mechanism cannot yet be considered as fully established.

5. The role of this reaction in the pathogenesis of the disease remains to be elucidated by further studies with additional sera of patients prior to transfusions.

We wish to thank Drs. Samuel M. Feinberg and Jacob Pruzansky of the Northwestern University Medical School, Chicago, Illinois, for their encouragement and cooperation in the early stage of this study; Dr. Ben Z. Werbin of the Children's Department, Hadassah Hospital, Tel Aviv, Israel, for supplying the human fava sera; Dr. Ervin Neter of the Children's Hospital, Buffalo, New York, for his cooperation and suggestions; Miss June Spohr for preparation of the extracts; and Mrs. W. L. Stafford, Jr., for financial support to the Allergy Laboratory.

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SERIAL INTRACUTANEOUS TESTING FOR BEE AND WASP SENSITIVITY

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A FEW years ago we reported our experiences with serious allergic reactions to bee and wasp stings.[1] We have now studied and treated over eighty cases, and our further total experience with them will be published elsewhere.[9] In fifty-five of these cases we have done serial intracutaneous testing, and the results are the subject of this article.

Studies by Benson,[2-4] Rockwell,[5] and Ellis and Ahrens[6] indicated that the sensitizing antigens were contained in the entire body of the insect and that extracts of the body minus the venom sac gave reactions equal to that obtained by an equivalent dilution of the venom itself. This was again confirmed by Perlman[7] in 1955. Loveless and Fackler,[8] studying a comparison of whole body and venom sac antigens on a precipitable nitrogen basis, reported venom to be from three and one-half to five times more active but made no comparison of the extracts with respect to the intensity of reactions in patients.

Material used consisted of glycerinated, 1-10 weight by volume, whole insect extracts* of mixed honey and bumble bee, paper wasp, hornet, and yellow jacket. Serial dilutions of the insect extracts from 1:100 up to 1:100,000,000 were prepared with buffered saline in the following manner: with separate sterile tuberculin syringes used for preparation of each dilution, 0.5 c.c. of extract was added to 4.5 c.c. of buffered saline in metal-capped rubber-stoppered vials and mixed thoroughly by rinsing the syringe three times from the contents of the vial before withdrawing the syringe. All four extracts were applied intracutaneously in dosages of 0.02 to 0.03 c.c. each, starting with the greatest dilution.

If a positive reaction with a minimum erythema diameter of 2.0 cm. occurred, no further testing of that insect was done. If no immediate reaction occurred in twenty minutes, the next lower dilution was applied; this procedure was continued until either each insect gave a positive test or until a dilution of 1:100 was applied. The first positive test (or tests) to appear during the serial testing was called the initial positive test and is referred to hereafter as the IPT. All testing on each patient was done at one session on the same day.

Originally, it was believed that a dilution of 1:1,000,000 was a more than safe level at which to begin testing. In 1955, however, a 13-year-old boy who

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*Prepared and supplied by Hollister-Stier Laboratories.

had had a shock reaction to a sting twenty-eight days previously was given intracutaneous tests with a 1:1,000,000 dilution of each of the four extracts. Within five minutes he developed large wheal and flare reactions to hornet and yellow jacket, generalized urticaria, and respiratory difficulty and rapidly went into shock with a blood pressure of 60/0 and stupor. He recovered.

This led us to change our procedure by starting with scratch tests using a 1:1,000,000 dilution and, if these were negative, then proceeding with intracutaneous testing at the same level. In 1956, however, using this method in a 12-year-old boy who had experienced a shock reaction to a sting eleven months previously, we obtained negative scratch tests at 1:1,000,000 with all four insect extracts, and yet when the same dilutions were given intracutaneously this boy had similar immediate skin reactions to hornet and yellow jacket and was in similar shock within five minutes. He recovered. This convinced us that scratch testing in these patients is unreliable and that intracutaneous testing or treatment started without tesing should begin at very high dilutions. Since that time, we have started all testing at dilutions of 1:100,000,000 and have experienced no further severe general reactions.

TABLE I. PATIENTS HAVING SERIAL INTRACUTANEOUS TESTS

	CHILDREN 3-14 YEARS	ADULTS 24-56 YEARS	TOTALS
All patients	42	13	55
Male	30	6	36
Female	12	7	19
Family and/or personal history of other allergy	32	11	43
Personal history of other allergy	14	4	18
No history of allergy	9	2	11

Of the fifty-five patients, forty-two were children between the ages of 3 and 14 years, while thirteen were adults between the ages of 24 and 56 years (Table I). This leaves the decade from 14 to 24 years with no cases. However, two of the younger adults had reactions during adolescence to establish

TABLE II. CLASSIFICATION OF CLINICAL SEVERITY OF STING REACTIONS

DESIGNATION	NO. OF PATIENTS	SIGNS AND SYMPTOMS
Slight general reaction (SLG)	5	Generalized urticaria, itching, malaise, anxiety
General reaction (G)	22	Any of above plus any two or more of following: generalized edema, constricted feeling in chest, wheezing, abdominal pain, nausea and/or vomiting, dizziness
Severe general reaction (SG)	17	Any of above plus any two or more of following: dyspnea, dysphagia, hoarseness or thickened speech, marked weakness, confusion, feeling of impending disaster
Shock reaction (SH)	11	Any of above plus any two or more of following: cyanosis, fall in blood pressure, collapse, incontinence, unconsciousness

the fact that this age group is not immune to insect sensitivity. There were thirty-six males and nineteen females. Forty-three, or 80 per cent, of the patients had a family and/or personal history of allergy and of these, eighteen or one-third had a personal history of other allergy. Eleven patients had no allergic history. Family history was unknown in one patient, an adopted child.

For purposes of evaluation, patients were classified, according to the clinical severity of their symptoms, into the following four groups (Table II):

The five patients in the first group, with what we called a slight general reaction, are designated by the initials *SLG;* they had urticaria, itching, malaise, and anxiety.

The second group of twenty-two patients had what we called a general reaction, designated by the initial *G*. They had, in addition to the above symptoms, any two or more of the following: edema, constriction in chest, wheezing, abdominal pain, nausea and vomiting, and dizziness.

The third group of seventeen patients with what we called a severe general reaction, designated by the initials *SG*, had, in addition to any of the above symptoms, any two or more of the following: dyspnea, dysphagia, thickened speech, weakness, confusion, and what was commonly described as a feeling of impending disaster.

The fourth group of eleven patients with what we called a shock reaction, designated by the initial *S*, had, in addition to any of the above symptoms, any two or more of the following: cyanosis, fall in blood pressure, collapse, incontinence, and unconsciousness.

TABLE III. INITIAL POSITIVE TESTS (IPT) IN FIFTY-FIVE PATIENTS

DILUTION OF TESTING MATERIAL	SLIGHT GENERAL REACTION (SLG)	GENERAL REACTION (G)	SEVERE GENERAL REACTION (SG)	SHOCK REACTION (SH)
1:100,000,000			*	
1:10,000,000		*	* *	
1:1,000,000		* *	* * * *	* * * *
1:100,000		* *	*	* * *
1:10,000		* * * * * *	* *	
1:1,000	* *	* * * * * * *	* * * *	*
1:100	*	* * * *	* *	* *
No positive tests	* *		*	*
Totals	5	22	17	11

Keeping the meaning of these initials in mind, if the IPT are tabulated according to the severity of the sting reaction, we see (Table III) that none of the milder cases of group SLG gave an IPT above 1:1,000 and that there is a definite trend to greater skin sensitivity as the sting reactions increase in severity. The two patients with shock reactions on testing at 1:1,000,000 almost certainly would have had positive tests at higher dilutions if they had permitted further testing. Apparently, the more severe the reaction, the greater is the

likelihood of skin sensitivity at high dilutions. Nevertheless, it must be noted that even in groups SG and SH, eleven of the twenty-eight patients with the most severe reactions showed no more skin sensitivity than did patients of the SLG group, and two of these patients with severe reactions had no skin sensitivity demonstrable by testing.

There was no significant difference in the spread of the IPT between children and adults, except that no adult gave an IPT above 1:1,000,000.

TIME INTERVAL FROM STING REACTION TO TESTING

After experiencing a sting reaction, patients and/or their parents are extremely apprehensive and in some instances present themselves within a few days of their reaction, demanding that something be done. In testing *these* patients, the tests surprisingly did not become positive until low dilutions were reached, although only one was in group SLG and two patients were seen only twenty-four hours after release from a hospital following shock reactions. Nine such patients were tested within ten days of their sting reaction. One patient gave no positive tests. There were four patients with an IPT at 1:100, three patients with an IPT at 1:1,000, one patient with an IPT at 1:10,000,000. It was only very recently, after the preliminary report was written, that the patient with an IPT at 1:10,000,000 was tested on the ninth day after the sting reaction.

We have speculated previously that, clinically, insect sensitivity closely resembles artificial animal anaphylaxis, and these results have raised the question as to whether this is confirmatory experimental evidence of this resemblance. It is known that if an artificially sensitized animal is shocked but not killed, there follows an approximate ten-day refractory period during which injection of a shocking dose of antigen fails to produce any reaction. I have been unable to find any report of the effect of this refractory state upon intracutaneous tests. If insect sensitivity is analogous, then these patients might be in a refractory period and have a latent sensitivity of much greater degree than their tests would indicate. If this is so, is this a good time to start desensitization treatment with high dosage, saving many steps in the inoculation schedule? Or would this be merely keeping a patient in a refractory state rather than providing actual desensitization? Further investigation is needed before these questions can be answered.

INCIDENCE OF ALLERGIC HISTORY

If the IPT are tabulated against allergic history (Table IV) twenty-five, or 90 per cent, of severe and shock cases had a family or personal history of allergy, while eighteen, or 69 per cent, of the less severe cases had an allergic history. Table IV also indicates that skin sensitivity at high dilutions occurred with much greater proportional frequency in the group with a history of other allergy than in the group without such a history.

There was no difference, either in the frequency of severe sting reactions or in skin sensitivity, between those having a family history of allergy and

TABLE IV. INITIAL POSITIVE TESTS (IPT) ACCORDING TO ALLERGIC HISTORY AND SEVERITY OF REACTION

DILUTION OF TESTING MATERIAL	FAMILY AND/OR PERSONAL HISTORY OF OTHER ALLERGY				PERSONAL HISTORY OF OTHER ALLERGY				NO FAMILY OR PERSONAL HISTORY OF OTHER ALLERGY			
	SLG	G	SG	SH	SLG	G	SG	SH	SLG	G	SG	SH
1:100,000,000			1				1					
1:10,000,000		1	1								1	
1:1,000,000		1	4	4			3	1		1		
1:100,000		1	1	3		1	1	1		1		
1:10,000		5	2			3						
1:1,000	1	6	3			2	2		1	1	1	1
1:100		2	2	2		1	1	1	1	2		
No positive tests	1		1	1					1			
Totals	2	16	15	10		7	8	3	3	5	2	1

SLG = Slight general reaction.
G = General reaction.
SG = Severe general reaction.
SH = Shock reaction.

those with an additional personal history of allergy except that the latter group contained none of the milder cases (Table IV). I believe that it is fair to say that patients with a family or personal history of allergy are more likely to have a severe sting reaction, and skin sensitivity is more likely to be present in the higher dilutions. However, patients with no allergic history can and do have severe reactions and exquisite skin sensitivity.

CROSS-SENSITIVITY

Turning to the insects involved, Table V indicates all IPT obtained with each insect. There was a total of 111 initial positive tests, or an average of two IPT per patient.

TABLE V. TOTAL INITIAL POSITIVE TESTS (IPT) BY EACH INSECT* ACCORDING TO SEVERITY OF REACTION

DILUTION OF TESTING MATERIAL		SLIGHT GENERAL REACTION (SLG)				GENERAL REACTION (G)				SEVERE GENERAL REACTION (SG)				SHOCK REACTION (SH)			
		B	W	H	Y	B	W	H	Y	B	W	H	Y	B	W	H	Y
1:100,000,000										1	1	1	1				
1:10,000,000						1	1	1		1	1	1					
1:1,000,000						1	1	1	2		1	2	3			5	5
1:100,000						1		1	2			1		1	1	1	2
1:10,000						4	1	5	3	1	1	1	2				
1:1,000		1	1	2	2	1	1	7	4	3	2	4	3			1	
1:100		1		1	1	4		2	1	1	1	1	1		2	2	2
Totals	111	2	1	3	3	12	4	17	12	7	7	11	10	1	3	9	9

*Key to insects is initial letter of bee, wasp, hornet, and yellow jacket.

Fourteen patients gave an IPT to one insect only—two with bee, two with wasp, six with hornet, and four with yellow jacket.

The remaining forty-two patients gave an IPT with two or more insects at the same dilution level. Hornet, yellow jacket, or a combination of the two, gave the greatest number of IPT. In fact, at least one of these two insects gave a positive test at the IPT level in all but four, or 92 per cent, of

those patients who gave any positive tests. In our opinion, this seems to suggest clinical confirmation of the experimental studies on antigenic relationships by Foubert and Stier.[10]

Eleven patients had no information that would help in identifying the insect causing the reaction sting. In four patients, the stinging insect was positively identified, and in each case the IPT included the identified insect. In the remaining forty patients in whom such identification was attempted, only twenty-four gave an initial positive test to the suspected insect. This would seem to indicate that identification is unreliable, and clinical experience has confirmed this.

Table VI summarizes the number and the percentages of positive tests obtained in each patient. A total of 163 positive tests were obtained.

TABLE VI. TOTAL POSITIVE TESTS TO FOUR SPECIES OF INSECTS

POSITIVE TESTS TO:	NUMBER OF POSITIVE TESTS	NUMBER OF PATIENTS	PER CENT OF PATIENTS
None	0	4	7.3
One insect	4	4	7.3
Two insects	20	10	18.2
Three insects	27	9	16.3
Four insects	112	28	50.9
Totals	163	55	100.0

This indicates that most patients (85 per cent) had multiple or cross-sensitivities, although there was no history of sensitizing stings by the various species. The degree of multiple sensitivity did not parallel severity of sting reaction.

DELAYED TUBERCULIN TYPE OF REACTION

Reactions of the delayed tuberculin type, with induration, itching, and erythema appearing in twenty-four to forty-eight hours, occurred in seventeen of the patients, three of whom had given no IPT. The delayed reactions were approximately equally divided between the two milder groups and the two groups with very severe reactions. In all cases the delayed tests were positive at the same level as the IPT, and in 70 per cent they were positive at a higher level. As yet, we do not understand the significance of these delayed tests.

SUMMARY

Exquisite sensitivity may be present in patients with systemic reactions to stings of bees or wasps, and great caution should be exercised in testing and/or treating these patients with insect extracts.

In general, the more severe the sting reaction, the greater is the skin sensitivity.

Patients having a family or personal history of allergy are more likely to have a severe sting reaction, and skin sensitivity is more likely to be present at high dilutions.

Multiple skin sensitivity was present in 85 per cent of these patients. Skin sensitivity to hornet or yellow jacket was present at the IPT level in 92 per cent of the patients giving positive tests.

Identification of the insect causing the sting reaction is unreliable unless the insect is brought in for examination.

Patients tested within ten days of their sting reactions had little skin sensitivity, except in one instance, and the question is raised as to whether these patients are in a refractory state.

CONCLUSION

The practical value of serial intracutaneous testing is in the determination of a safe level for starting polyvalent extract treatment. With it, many unnecessary inoculations may be eliminated in some patients. Without it, we believe that treatment should always be started at the 1:100,000,000 dilution level.

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300 LONGWOOD AVE.

ORAL PROPHYLAXIS AGAINST POISON IVY DERMATITIS WITH AQUA IVY TABLETS*

I. A Controlled Experiment and Preliminary Clinical Report

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INTRODUCTION

DERMATITIS venenata from poison ivy or poison oak has been a disturbing entity to many groups of persons who work or spend leisure hours in the countryside.

Protection from ivy poisoning may be developed by administering an ivy extract orally or parenterally. It was a custom among the American Indians[1] to eat the young green ivy leaves to prevent the ivy rash. The beneficial therapeutic effects of oral ivy dates back to the report of Dakin[2] in 1829. Warren[3] produced immunity by administering the extract orally in 1909. Schamberg[4] was the first to popularize the ingestion of the ivy resin which he dissolved in alcohol. Shelmire[5] employed the ivy resin in diluted corn oil and gave it by mouth in daily doses. There have been many reports[6-9] since then on the efficacy of oral ivy therapy. Spain and Cooke[9] showed that the alcoholic ivy resin was stable only in absolute alcohol and that the water present in even 95 per cent alcohol caused its rapid deterioration. Therefore, most oral tinctures of ivy rapidly lose their activity.

Gold and Masucci[7] treated ivy-sensitive patients with capsules containing from 0.5 to 10 mg. of the poison ivy oleoresin in corn oil. Daily treatment with increasing dosages up to fifteen capsules of the 10 mg. concentration through a total of forty-five days was reported. Seventeen of twenty-five ivy-sensitive patients responded favorably in controlled field exposure, but 80 per cent of these patients developed rather severe toxic reactions consisting of erythemas of the extremities, pruritus, vesicular dermatitis, or a papular rash. Kligman[10] has administered orally large dosages of pentadecylcatechol to ivy-sensitive persons, and marked toxic effects were reported.

The chief disadvantage of the liquid oral preparations is the danger of spreading the extract with its active principle upon the hands, lips, and

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*Alum-precipitated pyridine ivy extract. The Aqua Ivy tablets used in this study were supplied by Syntex Chemical Co., Inc., New York, New York.

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mouth, causing fresh lesions. Toxic side effects, such as pruritus ani, gastric upset, and vesicular dermatitis, have prevented general acceptance of this form of therapy.

Aqua Ivy, A.P.,* the alum-precipitated pyridine-ivy complex described by Strauss and Spain,[11] was the first active ivy preparation to be dispensed in aqueous solution. Successful clinical results have been reported[11-14] on over 500 ivy-sensitive persons treated with Aqua Ivy and virtually no toxic effects were observed. Since Aqua Ivy solved many problems relative to parenteral prophylaxis against poison ivy dermatitis, it was incorporated into tablet form for oral therapy.†

Dermatitis venenata is a problem with the men of the Second Coast Guard District. Poison ivy grows abundantly along the shores of the Mississippi River and its tributaries, and it is the duty of the Coast Guardsmen to clear these areas of overgrowth and other debris. Prior to the first study reported in this article (which was carried out in 1956), a survey was made to ascertain the incidence of poison ivy dermatitis in the District. Data was obtained on 153 men, all of whom were exposed to poison ivy to some extent. Of these, eighty-five men (56 per cent) had some degree of poison ivy dermatitis. The severity of the problem at hand necessitated some type of prophylaxis.

The Coast Guardsmen were stationed on ships which were sometimes away from their base for periods of up to four weeks or more, so that weekly parenteral prophylactic treatment was not feasible since there was no medical officer or pharmacist's mate on many of these ships. Oral prophylaxis against poison ivy was therefore attempted.

This article presents the results of a double-blind, controlled study performed in 1956 and a clinical study without controls performed in 1957. It evaluates Aqua Ivy Tablets as a prophylactic agent against poison ivy dermatitis.

EXPERIMENTAL PROCEDURE FOR CONTROLLED STUDY

One hundred forty-two men were included in the study. All were members of the United States Coast Guard who worked at clearing brush along the banks of the Mississippi River and its tributaries. The period of observation and tablet ingestion was from April 23, 1956, to Nov. 1, 1956.

Exposure to poison ivy was recorded for each man. In general, it may be said that almost all men were exposed; the average man was exposed two or three times per week to heavy poison ivy growth.

Two groups were formed in an entirely random fashion, and each ship had approximately the same number of men in each group. Group A took Aqua Ivy Tablets, and Group B took a placebo which had a chlorophyll base and looked quite like the Aqua Ivy Tablet.

*United States Patent No. 2,456,750. Trademark of Syntex Chemical Co., Inc., New York, New York.

†The suspension of Aqua Ivy for parenteral use is standardized so that the concentrate contains 15 mg. dry alum-precipitated pyridine-ivy per milliliter. Aqua Ivy Tablets are prepared from this concentrate.

The tablets given to Groups A and B were coded; no one aboard ship knew of the difference between tablets. Both groups received identical instructions and took their tablets in identical fashion. Tablet ingestion was supervised by the captains of each vessel every day. The following dosage schedule was used:

(1) *First week:* 0.06 mg. Aqua Ivy Tablet (or placebo) once daily.
(2) *Second week:* 0.06 mg. Aqua Ivy Tablet (or placebo) three times per day.
(3) *Third week:* 0.3 mg. Aqua Ivy Tablet (or placebo) once daily.
(4) *Fourth and fifth weeks:* 0.3 mg. Aqua Ivy Tablet (or placebo) three times per day.
(5) *Sixth week until end of study:* 1.5 mg. Aqua Ivy Tablet (or placebo) once daily.

Observation sheets were kept by the captain of the vessel on all subjects. The diagnosis of poison ivy dermatitis was usually made by the captain of the vessel, who, as a rule, had a great deal of experience with this entity. About 25 per cent of the diagnoses were confirmed by a physician. Cases were rated as mild, moderate, or severe according to the following criteria:

(1) *Mild:* Three or four small isolated blotches, none more than the size of a dime.
(2) *Moderate:* A definite rash on one or both extremities with only mild discomfort. Rash is not weeping and is not greater than 3 square inches in all.
(3) *Severe:* A rash covering a palm-sized area or more. Any rash that causes much discomfort and complaining. Any rash that is oozing noticeably.

RESULTS

Over-all Data.—The over-all results (that is, disregarding duration of observation and the point in the experiment at which the poison ivy dermatitis occurred) are outlined in Table I.

TABLE I. OVER-ALL RESULTS

GROUP	NUMBER OF MEN OBSERVED	NUMBER OF MEN CONTRACTING POISON IVY DERMATITIS
A (Aqua Ivy Tablets)	73	17
B (Control)	69	17
Totals	142	34*

*Over-all incidence 24 per cent.

These figures are not the definitive data. Cases of poison ivy dermatitis which occurred during the first six weeks of observation (and tablet ingestion) are included. Since it takes time to build immunity, it was decided in advance of the study to allow six weeks (the period of increasing Aqua Ivy Tablet

dosage) for the development of adequate levels of protection. Therefore, data which excluded all cases in both groups that occurred during the first six weeks in which the subject took the medication (or placebo) was tabulated (Table II).

Data Excluding First Six Weeks of Observation and Medication.—Table II includes all men observed for a period greater than six weeks and includes only those cases of poison ivy dermatitis occurring in either group after this initial six-week period.

TABLE II. RESULTS AFTER SIX WEEKS OF TABLET INGESTION

GROUP	NO. OF MEN OBSERVED	NO. OF MEN CONTRACTING POISON IVY DERMATITIS	INCIDENCE OF POISON IVY DERMATITIS (PER CENT)
A (Aqua Ivy Tablets)	65	4	6
B (Control)	57	12	21
Totals	122	16	—

$p = 0.035$.

$\frac{1}{2}p = 0.018$.

In the most strict psychologic experiment, a "p" or "½p" equal to 0.05 or less is highly significant. The "½p" value is used here since we predicted the direction of our results. Thus, the "½p" of 0.018 here means that there are 1.8 chances in 100 that our results are due to chance alone and are not due to the difference between Aqua Ivy Tablets and the placebo. Such a "½p" value is enormously significant. For medical experiments, with their many unpredictable variables, a "p" or "½p" of 0.20 is often considered to be significant. The lower the "p" value, the more conclusive the results.

These figures show at a very high and conclusive level of statistical significance the ability of Aqua Ivy Tablets to protect persons from poison ivy dermatitis. The difference of incidence when the two groups are compared shows that this is a valid finding and does not appear to be due to chance or any artefact.

Results in Subjects With Prior History of Poison Ivy Dermatitis.—Table III shows the data restricted to men who gave a previous history of poison ivy dermatitis.

TABLE III. RESULTS IN KNOWN IVY-SENSITIVE SUBJECTS

	OVER-ALL		EXCLUDING FIRST SIX WEEKS		
GROUP	NUMBER OBSERVED	NO. OF MEN CONTRACTING POISON IVY DERMATITIS	NUMBER OBSERVED	NO. OF MEN CONTRACTING POISON IVY DERMATITIS	INCIDENCE OF POISON IVY DERMATITIS (PER CENT)
A (Aqua Ivy Tablets)	18*	9	16	3	19
B (Control)	21*	12	17	8	47
Totals	39	21	33	11	—

$p = 0.188$.

$\frac{1}{2}p = 0.094$.

*The approximate equal number of men with a past history of poison ivy in the two groups verifies the evenness of the groups, as no attempt was made to control this in advance.

The figures which exclude the first six weeks of medication are here highly suggestive and may be considered statistically significant.

Results in Subjects Without Prior History of Poison Ivy Dermatitis.—Table IV shows the data for men with no past history of ivy dermatitis, many

TABLE IV. RESULTS IN MEN WITH NO PAST HISTORY OF POISON IVY DERMATITIS

	OVER-ALL		EXCLUDING FIRST SIX WEEKS		
GROUP	NUMBER OBSERVED	NO. OF MEN CONTRACTING POISON IVY DERMATITIS	NUMBER OBSERVED	NO. OF MEN CONTRACTING POISON IVY DERMATITIS	INCIDENCE OF POISON IVY DERMATITIS (PER CENT)
A (Aqua Ivy Tablets)	55	8	49	1	2
B (Control)	48	5	40	4	10
Totals	103	13	89	5	—

of whom were experiencing their first exposure to the ivy leaf. The results appear favorable.

Extent of Rash.—Table V shows the rating of all of the poison ivy dermatitis incidents which occurred, including repeated incidents in the same men.

Once again we shall review only the data after six weeks. Here it is seen that, of the four men in the group taking Aqua Ivy Tablets who contracted poison ivy dermatitis, three had mild cases. From this it is apparent that of the sixty-five men in this group only one man had more than a mild case of the poison ivy dermatitis (a 1.5 per cent incidence) and, of course, only four men had any rash at all. This contrasts with the control group in which four of the fifty-seven men had more than mild cases of poison ivy dermatitis—an incidence of 7 per cent.

TABLE V. INCIDENTS OF POISON IVY DERMATITIS RATED ACCORDING TO SEVERITY

	OVER-ALL					EXCLUDING FIRST SIX WEEKS				
GROUP	NO. OF MEN OBSERVED	MILD	MODERATE	SEVERE	TOTAL	NO. OF MEN OBSERVED	MILD	MODERATE	SEVERE	TOTAL
A (Aqua Ivy Tablets)	17	17	6	4	27	4	6	–	3*	9
B (Control)	17	18	10	2	30	12	11	6†	–	17
Totals	34	35	16	6	57	16	17	6	3	26

*All in the same man.
†In four different men.

Side Reactions.—There were six men (three in each group) who reported side reactions attributed to the tablets taken. These reactions were as follows:

1. Group A (Aqua Ivy Tablets)
   (a) "Makes me sick" (meaning unclear).
   (b) "Nausea."
   (c) A generalized poison ivy dermatitis was reported in a man with a history of recurrent poison ivy dermatitis over many years and a similar reaction to prior attempts at prophylaxis. Subject denied exposure. He stopped the tablets and the rash receded.
2. Group B (placebo)
   (a) "Headaches."

(b) "Drowsiness."

(c) One man contracted poison ivy dermatitis and felt that it was due to the tablets he was taking. He stopped the tablets and the rash receded.

No other reactions were reported. There is virtually no difference in the two groups.

CLINICAL STUDY

Experimental Procedure.—One hundred twelve men of the Second Coast Guard District participated under work conditions and exposure similar to those in the controlled study. The period of observation was from April 4, 1957, to Oct. 15, 1957. Aqua Ivy Tablets, 1.2 mg., were utilized exclusively in this study (there were no controls), and the following dosage schedule was followed by all subjects:

> ½ tablet (0.6 mg.) every other day for two weeks, then ½ tablet (0.6 mg.) daily for two weeks, and then 1 tablet (1.2 mg.) daily until 100 tablets had been ingested (three months).

Observation sheets were kept, as before, and the same rating scale for episodes of poison ivy dermatitis was used. At the end of the study, during the first week in December, patch tests* were performed on fifty-seven men.

TABLE VI. RESULTS OF CLINICAL STUDY

	NO. OF MEN CONTRACTING POISON IVY DERMATITIS								
	WEEKS 1 TO 6 ONLY					AFTER WEEK 6 ONLY			
	NONE	MILD	MODERATE	SEVERE	TOTAL	MILD	MODERATE	SEVERE	TOTAL
Men with a past history of poison ivy	41	1	1	–	2 (5%)	3	2	–	5 (12%)
Men with no past history of poison ivy	71	2	–	–	2 (3%)	1	–	–	1 (1.4%)
Totals	112	3	1	–	4 (4%)	4	2	–	6 (5%)

Clinical Results.—Table VI reveals that Aqua Ivy Tablets afforded complete protection against poison ivy dermatitis for 88 per cent of subjects with a past history of dermatitis after six weeks of tablet ingestion. The results are somewhat better in light of our rigid rating scale, since 93 per cent of the "sensitive" men achieved good protection (had only mild poison ivy dermatitis) over-all and 95 per cent received good protection after six weeks. The absence of a severe dermatitis in any subject is also worthy of note.

The results in those men with no past history of poison ivy (many of whom had never been exposed to poison ivy previously) are, as expected, somewhat better; no man had more than a mild dermatitis at any time.

*Patch tests were performed as follows: A fresh 10 per cent alcoholic ivy extract was prepared and one drop of a 1-10 dilution from a 26 gauge needle was placed on a ¼ inch disc, placed on the upper arm, and secured with a Band-Aid. After twenty-four hours the patch was removed, and the reaction was read twenty-four hours after this.

Results of the Patch Testing After Treatment With Aqua Ivy Tablets.—Since no patch testing was performed before tablet ingestion in this study, the results of patch testing after treatment are of limited value.

Twenty-seven men with no history of poison ivy dermatitis were patch tested, and all patch tests were negative. Fifteen men with a past history of poison ivy dermatitis were patch tested; thirteen were negative to patch test and two were positive.

Results of Patch Testing Men Not Treated With Aqua Ivy Tablet.—For comparison, men with a past history of poison ivy dermatitis and definite exposure, who did not take Aqua Ivy Tablets, were also patch tested. Of the fifteen men patch tested, twelve had positive patch tests and three had negative patches.

These results suggest two interesting ideas: first, that Aqua Ivy Tablets do not sensitize nonsensitive persons and, second, that Aqua Ivy Tablets may very well desensitize, as measured by patch testing, persons who are sensitive to poison ivy.

Side Effects.—Three men (2.5 per cent) reported very mild incidents of poison-ivy-like lesions in the web of the fingers during the first six weeks of tablet ingestion, at times when they were not exposed to poison ivy. In each case, the rash lasted about three days, caused no notable discomfort, and disappeared spontaneously without any sort of therapy. Tablet ingestion was not interrupted. No other side effects were reported.

Only thirty men participated in both the 1956 and 1957 studies. This small number does not provide sufficient material for meaningful analysis; the trends, however, are along the lines of our other data. Combining the clinical data of the two studies, we find that 86 per cent of the men with a past history of poison ivy dermatitis had complete protection against the dermatitis, while 98 per cent of the men without a history of the dermatitis were fully protected.

DISCUSSION

The results tend to show that Aqua Ivy Tablets offer immunity against poison ivy dermatitis. This conclusion appears warranted on the basis of the double-blind, controlled study which gave statistically significant results, as well as the clinical study in which 88 per cent of men with a past history of poison ivy dermatitis, under conditions of heavy exposure, had no dermatitis at all.

The present report differs from those in the literature in several important aspects: First, we have reported a controlled study performed in a "blind" fashion which produced statistically valid results. Second, we recorded exposure to the ivy leaf and have been able to verify heavy and fairly constant exposure. Third, we have set very stringent criteria with which to measure the clinical efficacy of Aqua Ivy Tablets; many would consider mild lesions, as we have defined them, as constituting excellent clinical results.

One problem must be acknowledged. Tablet ingestion in both studies was not preseasonal but coseasonal. During the first six weeks of the double-blind study (1956), thirteen men in the experimental group contracted the dermatitis, as compared with five men in the control group. Although the thirteen men in the first group did not develop a dermatitis again during the season, after more prolonged therapy, the early appearance of the dermatitis must be explained. One can only speculate at this time since a definite answer can come only from later studies which begin at least six weeks preseasonally. It is our impression, however, that many of these reports of "poison ivy dermatitis" were actually minor side effects—mild cases of dermatitis due to ingestion of the Aqua Ivy Tablets. To support this concept, we refer to the one subject in the controlled study and the three subjects in the clinical study who reported such mild lesions as "side effects" because they were certain that they had not been exposed to the ivy leaf. On the other hand, subjects being exposed would consider such lesions to be due to their contact with the leaf. Walzer[15] has shown that such an absorption is quite possible for molecules of this size.

There are minor drawbacks to the experimental procedure which should be mentioned. One is the lack of a physician's confirmation of the diagnosis of poison ivy dermatitis in 75 per cent of the cases. However, the captains are fairly competent in this regard, and their errors (if any) would have tended to be balanced out in the controlled study and toward an increased incidence of ivy dermatitis in the clinical study.

The relative absence of side reactions and the complete absence of toxicity are extremely important findings. No pruritus ani, mouth irritation, lesions of the hands due to handling the tablet, or significant gastrointestinal upset was reported. Incidental complete blood counts done on several subjects during the study were without abnormality. The key here seems to be the use in the Aqua Ivy Tablets of the pyridine-ivy resin complex which is alum precipitated and slowly absorbed. From this, it appears that previous side reactions which had rendered oral prophylaxis impractical were due to the native solubility and toxicity of the unaltered ivy resins in oil or alcohol. This impression certainly merits more detailed confirmation with more complete laboratory studies. These were not possible in these studies, but it should be noted that no clinical indication for such laboratory work appeared.

The duration of immunity remains unanswered and awaits further work.

SUMMARY

1. A controlled, double-blind study and a clinical study were performed in an effort to evaluate the use of Aqua Ivy Tablets as a means of providing oral prophylaxis against poison ivy dermatitis.

2. In the controlled study, the following pertinent results were obtained:

(a) Allowing a six-week incubation period for the formation of immunity, Aqua Ivy Tablets provided full protection for 94

per cent of the subjects who took the drug, so that only 6 per cent showed any form of poison ivy dermatitis. This is statistically a highly significant difference from the 21 per cent incidence of the dermatitis in the control group ($\frac{1}{2}p = 0.018$).

(b) Aqua Ivy Tablets protected 81 per cent of the men with prior history of poison ivy dermatitis, so that only 19 per cent of these men showed the dermatitis. This compares well with the 47 per cent incidence of poison ivy dermatitis in the "sensitive" men in the placebo group ($\frac{1}{2}p = 0.094$).

(c) There was only one man in the entire Aqua Ivy Tablet group who had more than a mild case of poison ivy dermatitis (1.5 per cent).

3. In the clinical study, after six weeks of tablet ingestion, 88 per cent of "sensitive" subjects had no dermatitis at all and 95 per cent of "sensitive" subjects had good results (that is, they had only minimal poison ivy dermatitis). In the entire group of 112 men, 95 per cent had complete immunity after six weeks and 98 per cent had good results.

4. No significant side effects appeared in the controlled study. In the clinical study, three "sensitive" subjects (2.5 per cent) reported a minimal and transient dermatitis in the web of the fingers which did not require therapy or necessitate interruption of tablet ingestion.

5. The over-all findings tend to show that Aqua Ivy Tablets provide a safe and effective means of oral prophylaxis against poison ivy dermatitis.

The work of Drs. G. P. Ferrazzano, United States Public Health Service Hospital, Chicago, Illinois, and John P. Schlemmer, United States Public Health Service Clinic, St. Louis, Missouri, in aiding in the collection of the data is gratefully acknowledged. We wish to express our appreciation to the men of the Second Coast Guard District for their participation and assistance.

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ALLERGY TO THE POLLEN OF THE COMMON SUGAR BEET (BETA VULGARIS)

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IT IS well known that pollens produced by a few members of the *Chenopodiaceae* and by most members of the related *Amaranthaceae* are significantly antigenic. The pollens of *Salsola kali* (Russian thistle), *Kochia scoparia* (burning bush), and the several species of *Amaranthus* (pigweed) are particularly bothersome, and the distribution of these plants is quite widespread. Other species, such as the many *Atriplexes* (saltbushes), *Sarcobatus vermiculatus* (greasewood), *Allenrolfea* (iodine bush or pickleweed), *Suaeda* (inkweed or sea blite), and *Bassia hyssopifolia* (smotherweed), produce pollens which probably are clinically antigenic in certain instances.

The prevailing concept of the antigenic relationship of the various species of the chenopod and amaranth families was stated recently by Wodehouse[1]: "It has long been known that the pollens of the various species of these two families cross-react to a large extent, showing a high degree of antigenic similarity both within the family and across the family boundaries." Wodehouse's recent antigenic analyses with gel diffusion techniques indicate, however, that there is a remarkable degree of heterogeneity within the *Chenopodiaceae* and that the antigens of the *Amaranthaceae* are probably identical for each of the several members of that family.

The common sugar beet (Fig. 1), *Beta vulgaris,* is a member of the *Chenopodiaceae*. It belongs to the subtribe *Beteae* and to the tribe *Cyclolobeae*.[2] Horticultural varieties of *Beta vulgaris* include the sugar beet, garden beet, fodder beet, and chard or foliage beet (spinach beet). In locations where it is cultivated strictly for the production of the vegetative sugar-containing beet, it has no antigenic significance, since the plant does not pollinate. In certain areas of the United States, however, the plant is grown entirely for the purpose of seed production, and the pollens produced there should be capable of sensitizing a certain number of persons.

Allergy to the pollen of the sugar beet was first reported by Dutton[3] in 1938. Small acreages of the plant had been grown experimentally in the Mesilla Valley of New Mexico and Texas for several years prior to her report. The plant was grown in abundance in 1935, as a result of which large quantities of sugar-beet pollen became airborne in the spring of 1936. During the ensuing two years, Dutton observed approximately 100 patients with respiratory allergy which seemed clearly due to the pollen of *Beta vulgaris* and with positive skin tests to that pollen. It was Dutton's feeling that, "the antigenicity of the pollen is maximal and that treatment is less successful than for the other pollens

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of this region." Cultivation of sugar beet for seed production was discontinued in the El Paso region in 1940; therefore, this pollen is no longer a problem in that area.[4]

In 1939, Phillips[5] reported similar findings in the Phoenix area. Approximately 1,800 acres of sugar beets were planted in Phoenix in 1936, and by 1938 there were 5,500 acres, all of which went to seed. Apparently there were no reports of definite manifestations of clinical sensitivity to beet pollen in 1936 and 1937, but in 1938 there was a sharp increase in the number of hay fever sufferers at the time that *Beta vulgaris* was pollinating. Many positive skin test reactions to sugar beet pollen were found and there seemed to be a great deal of cross-reaction with pollens of other species of the *Chenopodiaceae*. Phillips found,

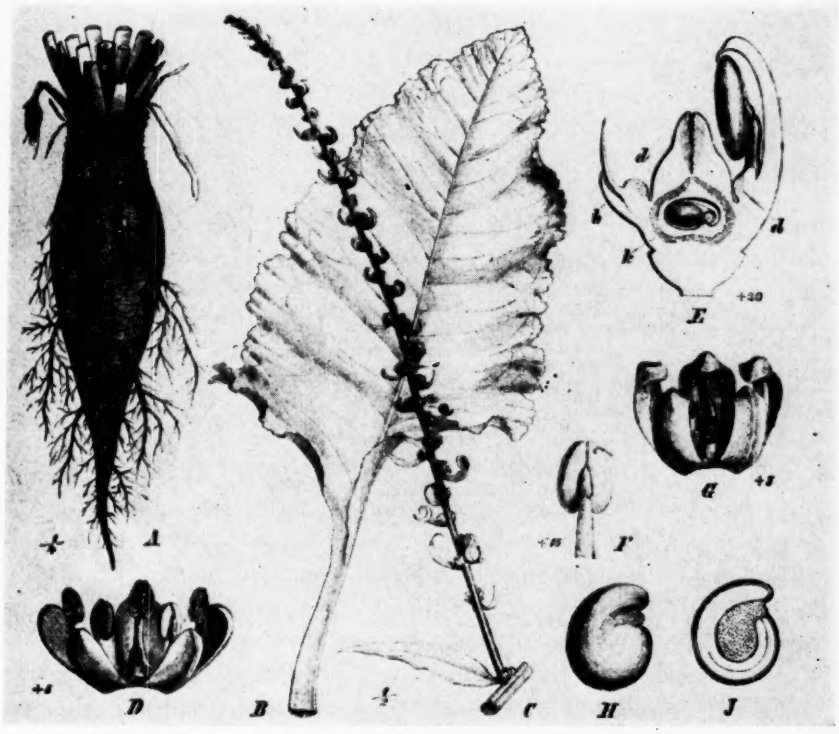


Fig. 1.—*Beta vulgaris* (common sugar beet). *A*, Root (sugar beet); *B*, leaf; *C*, part of the inflorescence; *D*, male flower; *G*, female flower. (From Engler-Prantl: *Natürliche Pflanzen-Familien*, 1894.)

however, that treatment directed "against the pollens of amaranth and Russian thistle did not provide a defense against sugar beet pollen." He concluded that "the sugar beet has, in addition to the group atopen common to the Chenopodiales, a highly potent specific atopen of its own." In a recent communication, Phillips[6] recalled his earlier experiences: "When it first appeared in our local pollen picture, I collected it personally. Emerging from rows higher than my head, I would be covered from head to foot with the pollen." This presents a vivid description of the voluminous quantity of pollen produced at that time by the plants in that area.

Sugar beet was cultivated for seed in the Safford area of Arizona (southeast of Phoenix) in the 1930's[7, 8] but its cultivation was discontinued, ostensibly because of the widespread protest about its allergenic qualities. Gatterdam[7] cites as an example of the strenuous objection to sugar-beet pollen on the part of some residents the deliberate planting of "stock beet" near the commercial fields, in the hope of causing cross-pollination with the commercial sugar beet and thus producing an inferior seed. Apparently, the pollen continues to have clinical significance in the Phoenix area, causing difficulty particularly in the spring. Vivian[8] feels that it is a very potent sensitizer, and he undertakes hyposensitization when indicated.

Sugar beet is grown commercially for seed production in numerous areas in the United States. These data in Table I show that the greatest acreage is in Arizona, which has approximately six times as much area under cultivation as Utah.

TABLE I

STATES	ACREAGE HARVESTED IN 1956	YIELD PER ACRE (POUNDS)
Arizona	2,061	2,760
California	674	3,233
Oregon	255	2,470
Utah	340	967
Other states	4	532

O'Toole[9] has found the pollen of sugar beet grown in Arlington and Hemet, California, to be as toxic as English walnut and olive. He is "continually surprised at the frequency of clinically significant skin tests to sugar beet in these areas, and in many instances this is the only pollen to which patients react." O'Toole has approximately sixty patients under treatment for allergy to sugar-beet pollen, which is comparable to the figure given by Phillips.[5]

Sugar beet is grown for seed production in appreciable amounts in Europe, particularly in Holland, England, Sweden, and Germany. Owen*[10] kindly communicated with four sugar-beet-plant investigators in England, Holland, and Sweden to inquire about the general frequency of allergy to sugar-beet pollen in those countries. These investigators, some of whom have had as much as twenty years' experience in the cultivation of *Beta vulgaris,* cited only five known instances of allergic rhinitis clearly due to sugar-beet pollen among the many persons constantly working closely with the plants. Two of these instances occurred in Sweden, two in Holland, and one in England. While these informal reports bear no statistical significance, and we have not obtained opinions from allergists in those countries, one is tempted to infer that sensitization to the pollen of *Beta vulgaris* probably is less common in England, Holland, and Sweden than in Phoenix, Arizona, or in the Hemet-Arlington region of California.

Reports received by Owen from sugar-plant investigators in this country indicate, in a most general way, that allergy to sugar-beet pollen is not prevalent in the beet-seed-producing areas of Salem, Oregon; East Lansing, Michigan; and

*F. V. Owen, Ph.D., Principal Geneticist, Sugar Beet Experimental Station, United States Department of Agriculture, Crops Research Division, Salt Lake City, Utah.

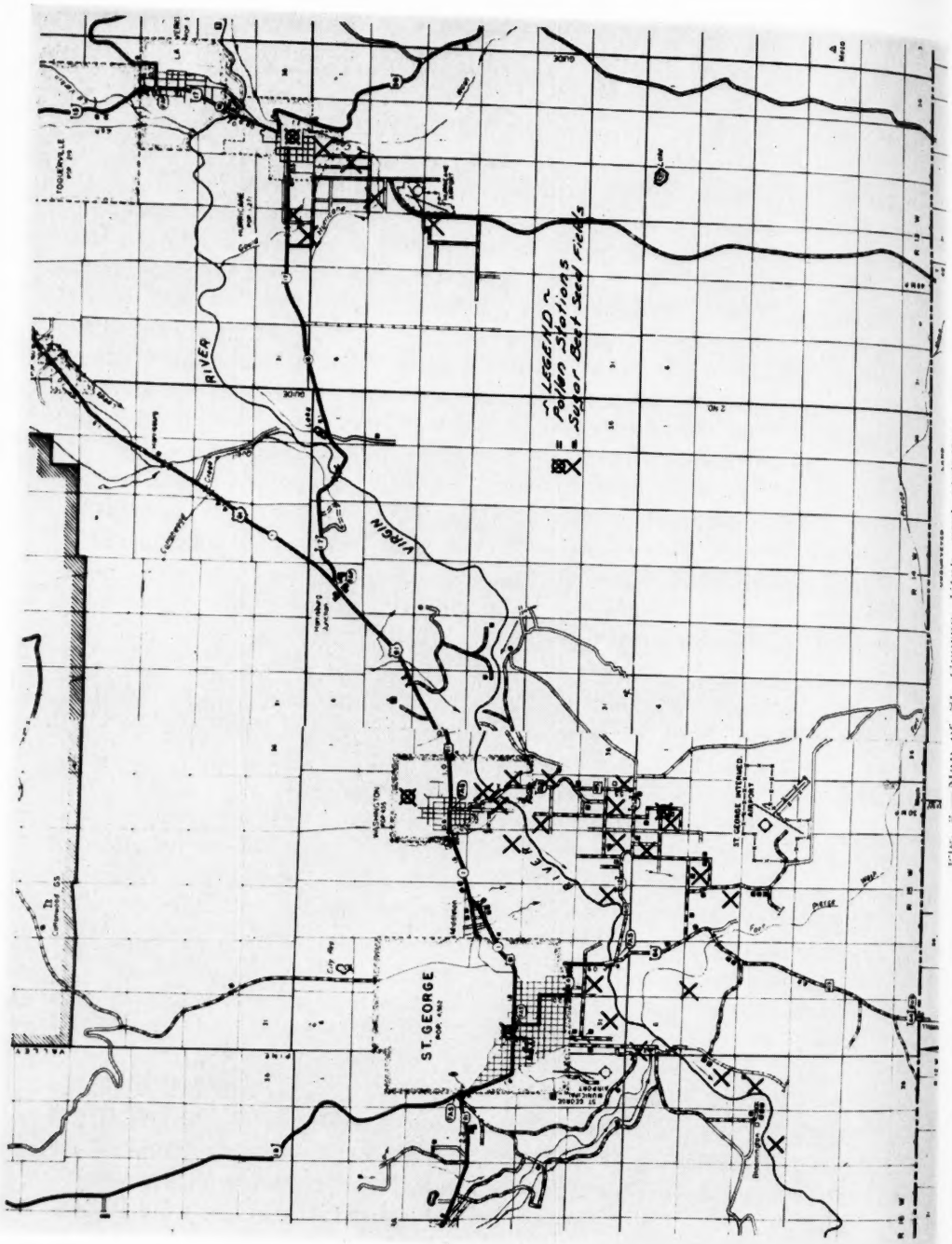
Rocky Ford, Colorado. Other reports to Owen from investigators at Beltsville, Maryland, and Phoenix, Arizona, indicate, however, that such allergy is not uncommon in those two areas.

H. Savitsky of the Sugar Beet Experimental Station, Salt Lake City, Utah (formerly of Kiev, Russia), has been an active sugar-plant investigator for thirty years. It is interesting that she had not heard of a single instance of allergic rhinitis or asthma caused by sugar-beet pollen in Russia, Poland, or Germany; nor had she, herself, experienced any such symptoms prior to 1955. She has been in intimate contact with pollen at Salt Lake City since 1948, but it is only since 1955 that she has had classical allergic rhinitis symptoms when near the pollinating plants. A few other investigators in the local Sugar Beet Experimental Station, including Owen himself, have become allergic to the plant and repeatedly have typical nasal symptoms when working closely with the pollinating sugar-beet plants. It is not known whether soil or other differences in such widely separated locations can account for significant variation in the antigenicity of the plant. Cooke[11] doubts that it is possible to alter the chemistry of pollen appreciably with respect to its antigenicity as a result of different chemical compositions of the soil. Lowell[12] is also skeptical as to the possible effect of geographic location on the antigenicity of plant products.

Hampton[13] agrees essentially with Cooke and Lowell. He feels, however, that investigation in this field is warranted and suggests doing neutralization in passive transfer with whole extracts of beet pollen obtained from different soils, using a single sensitive serum.

Production of sugar-beet seed in Utah is largely limited to the extreme southwestern corner of the state and extends into southeastern Nevada. Cultivation of sugar-beet plants for seed was begun in that area in 1931, at which time only six acres were planted in the Washington fields near St. George. The total yearly average since 1935 has been approximately 500 acres, but this has varied considerably from year to year. While there are many localities in other parts of Utah and in Idaho where the plant is cultivated strictly for the production and harvest of the sugar beet itself, the climate of the southwestern corner of the state has been most favorable for seed production.

In the Utah and Nevada areas the beet seed is planted early in September, and the plants usually attain a height of only 5 to 7 inches before frost. The following spring the plants produce seed stalks that attain a height of 5 to 7 feet by early summer. Prior to 1956, the pollination of the plants in late May or early June invariably filled the air with a sickening-sweet smell that could be detected for long distances. Since 1956 most of the varieties grown have been commercial hybrids. For production of these hybrids, approximately 95 per cent of the plants are male sterile monogerm beets that produce no pollen. About 5 per cent of the plants are pollinators, which produce sufficient pollen to fertilize the male steriles. This cross-fertilization produces a vigorous hybrid variety. The seed is usually threshed in August and is cleaned in September and October. Crop rotation is practiced, and a given field is used for beet-seed production no oftener than once every five years.


LEGEND
= Pollen Stations
= Season Beef Study Fields
RIVER
VIRGIN
ST. GEORGE
ST. GEORGE INTERNATIONAL AIRPORT

In 1955 the curiosity of one of us (G. A. P.) was aroused by frequent reports of respiratory allergy due to sugar-beet pollen in the St. George area. This motivated the establishment of three pollen-counting stations in May and June, 1955, in and near St. George and Washington, Utah, as shown in Fig. 2. United States Weather Bureau wind data for southwestern Utah were studied, and the stations were situated to reflect the volume of sugar-beet pollen blown on St. George and surrounding areas. The sugar-beet field station was placed on a fence immediately adjoining a vigorous acreage of sugar-beet plants in the Washington Fields section near St. George. A station was placed on the roof of a tall building in St. George to measure the amount of pollen blown into the town itself. The station in Washington, Utah, was approximately 7 miles downwind from the Washington Fields. Table II shows the pollen counts (in grains per cubic yard of air) from May 19 through June 13, 1955. It would seem that relatively little pollen was carried over the town of St. George, although there was somewhat more effective dispersal of the pollen downwind to Washington, Utah.

TABLE II. SUGAR-BEET POLLEN COUNTS (IN CUBIC YARDS OF AIR), 1955

DATE	ST. GEORGE	SUGAR BEET FIELD	WASHINGTON
May 19	0	301	0
May 20	0	29	3
May 21	0	128	3
May 22	0	44	5
May 23	0	61	3
May 24	5	--	0
May 25	0	82	3
May 26	5	668	3
May 27	0	216	0
May 28	0	1,328	2
May 29	0	1,054	2
May 30	0	1,447	9
June 1	0	541	0
June 2	2	425	0
June 3	3	7	2
June 4	12	75	0
June 5	41	190	0
June 6	0	187	0
June 7	0	31	0
June 8	0	17	0
June 9	0	85	0
June 10	2	583	0
June 11	3	71	0
June 12	0	182	0
June 13	0	54	3

In 1956 one of us (G. A. P.) became interested in sampling public opinion concerning allergy to sugar-beet pollen in a small community in southwestern Utah. Questionnaires were distributed to every family in Hurricane, Utah, a town of 1,271 persons, located 18 miles northeast of St. George in the sugar-plant region. A high percentage of the families in Hurricane are, or have previously been, concerned with the cultivation of sugar-beet seed. Completed questionnaires were returned by 919 persons, representing 228 families. Twenty-four per cent of these persons indicated that they have a major allergy. If the

answers given in the questionnaires were reasonably accurate, we must conclude that the incidence of allergy in this southwestern Utah community is somewhat higher than the national average. Twenty per cent of these persons limited their symptoms to the respiratory tract, while 12.6 per cent blamed sugar-beet pollen for part or all of their trouble. Fifty-one of the 181 persons (28 per cent) complaining of respiratory tract allergy incriminated sugar-beet pollen as the sole cause of their trouble. From the layman's standpoint, then, it would seem that sugar-beet pollen in southwestern Utah is suspected of causing more than its share of allergic rhinitis and asthma.

TABLE III. RESULTS OF SKIN TESTING IN THIRTY-NINE PATIENTS WHO GAVE HISTORY OF ALLERGY TO SUGAR-BEET POLLEN

SUGAR BEET	WINGSCALE	PIGWEED	RUSSIAN THISTLE
+	−	−	−
+	+	+	+
+	−	+	+
+	−	+	+
+	−	+	+
+	−	+	+
−	−	−	−
−	−	+	−
−	−	−	−
−	−	+	+
−	−	−	+
+	+	+	+
−	−	−	−
+	−	+	+
+	−	+	−
−	−	−	−
−	−	−	+
+	+	+	+
+	+	+	+
−	−	+	−
−	−	−	−
+	+	+	+
+	−	−	−
+	−	−	+
−	−	−	+
+	+	+	+
−	−	+	−
−	+	+	+
−	−	−	+
−	−	+	+
−	−	−	−
+	−	+	−

It would have been completely impractical to attempt to skin test each of the 1,271 inhabitants of Hurricane, Utah. It was felt, however, that a cross section of that population might be tested to some advantage, and on Jan. 19, 1957, one of us (D. A. M.) skin-tested fifty-eight of the persons who had answered the questionnaire. Thirty-nine of these were selected because they

blamed their respiratory allergy entirely on sugar-beet pollen. Nineteen were selected as controls whose histories indicated no trouble from sugar-beet pollen, but definite respiratory allergy from other causes. We would have preferred a total of thirty-nine controls, but many who had previously agreed to be tested failed to appear. Each of the thirty-eight patients was tested intradermally to *Beta vulgaris, Atriplex canescens* (wingscale), *Amaranthus retroflexus* (rough pigweed), *Salsola kali* (Russian thistle), and a saline control. The *Beta vulgaris* pollen, obtained from a commercial laboratory, was collected in the vicinity of Rocky Ford, Colorado. Comparative data are presented in Tables III and IV.

TABLE IV. RESULTS OF SKIN TESTING IN NINETEEN PATIENTS WITH SEASONAL RESPIRATORY ALLERGY AND NEGATIVE HISTORY FOR ALLERGY TO SUGAR-BEET POLLEN

SUGAR BEET	WINGSCALE	PIGWEED	RUSSIAN THISTLE
+	+	+	+
−	+	−	−
−	−	+	+
−	−	−	−
+	−	+	+
−	−	−	−
+	+	+	+
−	−	+	−
+	−	−	+
+	+	−	+
−	−	+	−
−	−	−	+
−	−	−	−
+	+	+	+
−	−	−	+
−	−	+	+
+	+	+	+
−	−	−	−
+	−	+	−
Control −	−	−	−

In the group of thirty-nine patients who felt that sugar-beet pollen caused allergic symptoms, only nineteen (48 per cent) showed a positive reaction to *Beta vulgaris*. Fourteen (74 per cent) of the nineteen persons who showed such positive reactions to *Beta vulgaris* also reacted to Russian thistle, fifteen (79 per cent) reacted to pigweed, and nine (47 per cent) reacted to wingscale. Seven (35 per cent) of the twenty persons who showed negative reactions to sugar-beet pollen failed to react to each of the other test antigens.

In the group of nineteen controls who suffer from respiratory allergy but feel that they are not bothered by sugar-beet pollen, the following data are pertinent:

1. Eight (42 per cent) exhibited positive reactions to sugar-beet pollen.
2. Fifteen (79 per cent) exhibited positive reactions to one or another of the test antigens.
3. Each of the eight who showed positive reactions to sugar-beet pollen reacted to at least one of the other test antigens.

Considering the entire group of fifty-eight persons tested, it is apparent that eleven (19 per cent) failed to react to *any* of the chenopod-amaranth antigens. Probably the respiratory tract allergies in these thirty cases are caused by pollen antigens not used in this particular testing, such as grasses, English plantain, and others. Focusing attention upon the forty-seven persons who reacted to one or more of the chenopod-amaranth antigens, we find the following:

1. Twenty-seven (57 per cent) reacted to sugar-beet pollen.
2. Thirty-five (75 per cent) reacted to Russian thistle.
3. Thirty-three (70 per cent) reacted to pigweed.
4. Seventeen (36 per cent) reacted to wingscale.

These figures indicate that sugar-beet pollen has an average capability to sensitize, as compared with three related pollens. These data further indicate

TABLE V. POLLEN COUNTS (IN CUBIC YARDS OF AIR), HURRICANE, UTAH, 1957

DATE	SUGAR BEET	OTHER CHENOPODS	PECAN	GRASSES	PINE
June 1	0	0	10	13	0
June 2	0	0	3	3	1
June 3	0	0	7	5	1
June 4	0	0	2	11	1
June 5	2	0	0	5	1
June 6	0	0	1	5	0
June 7	9	0	0	2	1
June 8	0	0	0	0	0
June 9	2	0	0	0	0
June 10	0	2	0	2	0
June 11	3	9	1	2	0
June 12	2	2	0	3	2
June 13	0	3	1	2	0
June 14	3	2	0	2	2
June 15	2	2	0	2	0
June 16	0	3	0	5	1
June 17	0	0	0	2	2
June 18	2	2	0	3	1
June 19	10	5	0	11	9
June 20	0	0	0	0	0
June 21	0	2	0	0	0
June 22	2	2	0	0	3
June 23	0	3	0	0	2
June 24	0	2	0	0	3
June 25	2	7	0	0	12
June 26	2	3	0	0	4
June 27	0	0	0	0	3
June 28	2	0	0	0	1
June 29	3	5	0	5	0
June 30	0	0	0	0	1
July 1	0	0	0	0	0
July 2	3	2	0	3	9
July 3	5	3	0	0	6
July 4	0	0	0	2	4
July 5	0	0	0	0	2
July 6	0	0	0	0	0
July 7	0	2	0	2	0
July 8	0	7	0	6	2
July 9	0	0	0	3	0
July 10	0	3	0	0	0
July 11	0	2	0	0	0
July 12	0	0	0	0	2

that in many instances sugar-beet pollen in southwestern Utah is blamed for respiratory allergy symptoms which in all probability, are caused by other pollens.

While there is some indication that sugar-beet pollen is less antigenic in southwestern Utah than in Phoenix, Arizona,[5] or in the vicinity of Riverside, California,[9] there is not a good basis for comparison between our work and that of O'Toole, Phillips, and Dutton. The reports made by Phillips and Dutton referred to apparently high sensitization rate as well as to unusually severe symptoms. However, their studies were not oriented toward determining the approximate incidence of allergy to sugar-beet pollen. O'Toole's observations are made on patients seen in consultation for diagnosis and treatment; they point to considerable specificity in the antigenicity of *Beta vulgaris* and also indicate unusually severe symptoms from the pollen. Our observations, on the other hand, were made on a group of persons who were not our patients. This precluded detailed histories; hence, we knew almost nothing about the relative intensity of their symptoms. Our tests indicate, however, that in our series sugar-beet pollen was less antigenic than Russian thistle and probably was less antigenic than pigweed.

Pollen counts were instituted in Hurricane, Utah, beginning April 6, 1957, and continuing through the month of September, 1957. Pollen counts from June 1 through July 12, 1957, are summarized in Table V. The relatively low pollen counts for *Beta vulgaris* are partly the result of a small sugar-beet crop in that area in 1957, but principally they are due to the planting of male sterile hybrid beets in southwestern Utah during the past three growing seasons. The substitution of the male sterile monogerm hybrids probably reduces the amount of sugar-beet pollen to 5 per cent of the original figure encountered in 1955. With the change to production of the male sterile hybrids, it is quite likely that in future years there will be insufficient amounts of *Beta vulgaris* pollen in southwestern Utah to be of any particular antigenic significance.

SUMMARY AND CONCLUSIONS

1. A study has been made of allergy to sugar-beet pollen *(Beta vulgaris)* in southwestern Utah.

2. The entire town of Hurricane, Utah, in the sugar-beet-cultivation area, was surveyed by questionnaire. Answers to the questionnaire indicated that a large number of persons in that community incriminate sugar-beet pollen as the cause of respiratory allergy symptoms. Intradermal tests done in fifty-eight persons indicated, however, that in 52 per cent of the cases, sugar-beet pollen is blamed for symptoms which must be caused by other antigens.

3. The sugar-beet pollen used in our intradermal tests exhibited average sensitizing ability when compared with related pollens. Considerable cross reaction to the related pollens was exhibited.

4. Information obtained from a number of sugar-plant investigators indicates that allergy to sugar-beet pollen may be comparatively uncommon in

Europe and Russia. Whether this can be explained on the basis of a difference in antigenicity due to soil composition or other factors is unknown. Further investigation of this question may be warranted.

5. The amount of sugar-beet pollen in southwestern Utah has been greatly decreased since 1956 by the introduction of male sterile hybrids in which only 5 per cent of the plants in the seed fields produce pollen.

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535 EAST FIRST SOUTH ST.

DELAYED HYPERSENSITIVITY IN MICE

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WHEREAS delayed hypersensitivity in its best-known form, tuberculin allergy, has been easy to induce and detect in guinea pigs, rabbits, man, and the ungulates, failure to detect it in mice and rats has been so common that these species generally are thought unable to develop it. The strongest point supporting this thought is that a classic delayed type of skin reaction has never been demonstrated in members of either species. Careful review of published experiments, however, reveals that rats and mice can be so sensitized and their reactions detected if special precautions and techniques are employed. A discussion of the most significant among these experiments will be presented. This discussion will be preceded by descriptions of experiments in white mice offering new evidence to supplement that of other workers and utilizing a novel technique which measures the reactions objectively where they appear to occur most intensely—in the lungs.

MATERIALS AND METHODS

CF#1 male and female white mice, 4 to 6 weeks old, were purchased for these experiments and kept in small groups. They were fed Rockland mouse pellets and water ad libitum and cracked corn and whole wheat twice a week.

Both vaccinating and challenge antigens were suspended in physiologic sodium phosphate buffer at pH 7.4. When water-in-oil (w-o) emulsions were employed for vaccination, they consisted of five parts buffer vigorously mixed with ten parts light mineral oil and two parts mannide mono-oleate (Arlacel A).

Two human varieties of tubercle bacilli, cultured on slightly modified Kirchner liquid medium, were utilized both for vaccination and for challenge. One was the avirulent strain H37Ra, whereas the other was the virulent strain Erdman. Bovine gamma globulin (Cohn Fraction II), bought from Nutritional Biochemicals Corporation, Cleveland, Ohio, was stored as a powder at 4° C. until used. Tuberculoprotein, also stored as a dried powder, was prepared from Seitz-filtered, unheated culture medium supporting growth of H37Ra bacilli by precipitation with 60 per cent w/v ammonium sulfate followed by dialysis against distilled water and then lyophilization. A crude lipid adjuvant was extracted from heat-killed Erdman bacilli with acetone and purified somewhat by evaporating the acetone and redissolving the essential lipid from the remaining aqueous residue suspension with 3 volumes of low-boiling-point petroleum ether. This solvent then was evaporated and the lipid stored in a stoppered amber bottle until used.

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Bacillary suspensions were made from ten- to fourteen-day cultures by homogenization with a fine-grade Ten Broeck tissue grinder at several times the final concentration required and then appropriately diluted. Bacillary concentrations are expressed as milligrams of blotted but moist bacilli.

Ordinarily, mice received two vaccinations one week apart and were challenged two weeks after the second, but this procedure was varied occasionally, as noted below. Challenge was by intravenous injection of 0.2 ml. phosphate-buffered bacillary suspension, considerable care being taken to ensure challenge uniformity from animal to animal. Thus, injections were made with a 0.5 ml. tuberculin syringe and 26 gauge needle, only two doses being withdrawn at one time from a frequently shaken vaccine bottle of stock suspension. Precautions also were taken to minimize nonspecific lung damage by the injected material.

At various intervals after challenge, mice were killed with ether vapor and their lung densities were determined by a technique described elsewhere.[1, 2] Essentially, the technique consists of extracting the entire lungs from a freshly killed mouse and dropping them into each of a series of nonpolar solvent baths, progressively increasing in density by increments of 0.02 Gm. per milliliter until a density which floats them is reached. The mean of this and the density of the preceding bath is recorded as the lung's density. Whereas normal mouse lung density ranges from just below 0.68 to 0.70, lungs from mice undergoing delayed hypersensitivity reactions usually equaled or exceeded a density of 0.73 in the present experiments.

EXPERIMENTS AND RESULTS

During immunization experiments in which H37Ra-vaccinated mice were challenged intravenously with living Erdman tubercle bacilli and their lung densities were determined over the next few days to follow development of tuberculosis, attention was drawn to small but apparently significant transitory lung density increases occurring in vaccinated mice from one to three days following challenge. These increases were not seen among unvaccinated mice, and they could be distinguished from later progressive and greater lung density increases due to developing tuberculosis. An experiment set up to study the reaction sequence illustrates these observations.

Participants in one mouse group received two weekly intraperitoneal injections of 0.25 mg. living H37Ra bacilli in 0.1 ml. w-o; those in the second group were injected identically but with an emulsion containing no antigen. Both groups were challenged one week after the second injection with 0.03 mg. of living Erdman bacilli in 0.2 ml. buffer, and lung densities of three or four mice from each group were measured one, six, and twenty-four hours and two, three, four, and six days after challenge.

Results from this experiment, plotted in Fig. 1, show that during the first twenty-four hours after challenge lung densities remained normal for both groups of mice, except for a spuriously high first sampling from the immunized group. In this group, one of four mice had a lung density of 0.81, whereas

the other three had lung densities of 0.71. Later investigation showed this single high reading to have no allergic significance. Deviations of this kind did not occur among mice of any other sampling in this experiment, although they have occurred infrequently among samplings from occasional other experiments.

From Fig. 1, it is obvious that H37Ra-vaccinated mice reacted to tubercle bacilli injected intravenously with lung density increases occurring between the second and fourth days after challenge, while lung densities among w-o-vaccinated mice challenged identically remained normal until about the sixth day, when they began to increase as a manifestation of tuberculous infection. By this time, lung densities in the immunized mice had returned to the normal range.

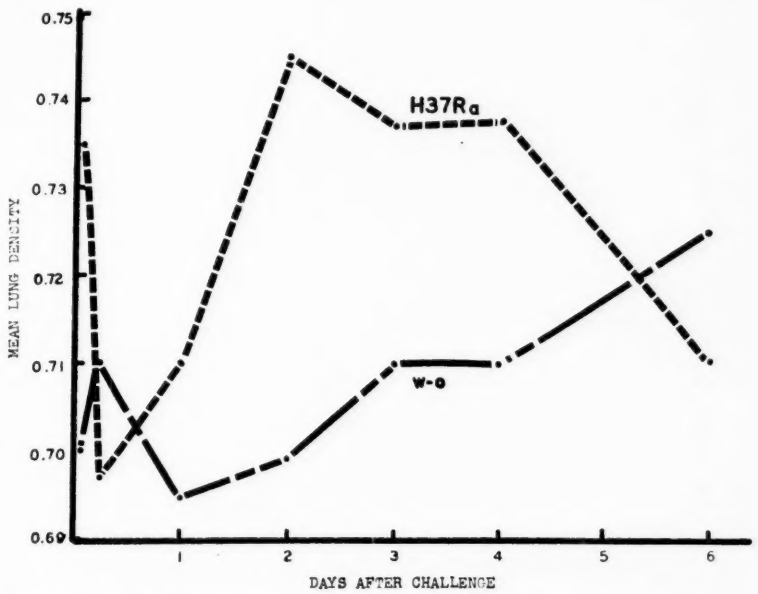

Fig. 1.—Mean lung densities of vaccinated (H37Ra) and control (w-o) mice determined at various intervals after their intravenous challenge with 0.03 mg. living tubercle bacilli (Erdman strain).

Neither group of mice gave any overt evidence of either anaphylactoid or systemic delayed hypersensitivity shocks at any time after challenge. However, whereas the lungs of challenged control mice were a light pink, characteristic of normal mouse lungs, until the last sampling, lungs from the test mice sampled two, three, and four days after challenge showed varying degrees of hemorrhagic congestion with numerous minute hemorrhagic spots scattered throughout them. A correlation appeared to exist between increased lung density and increased lung redness. Such lung pathology seems to be found commonly in tuberculin-shocked mice.[3, 4]

This experiment was designed primarily to establish the delayed time relationship between challenge and the increased lung density reaction in sensitized mice as compared with unsensitized mice. Although virulent bacilli were

employed for challenge, the experiment suggested that the lung density reaction in sensitized mice was not due to concurrently developing infection. The frequency with which samplings were made and the consistency of findings counterbalance the sampling smallness in showing that these reactions were the

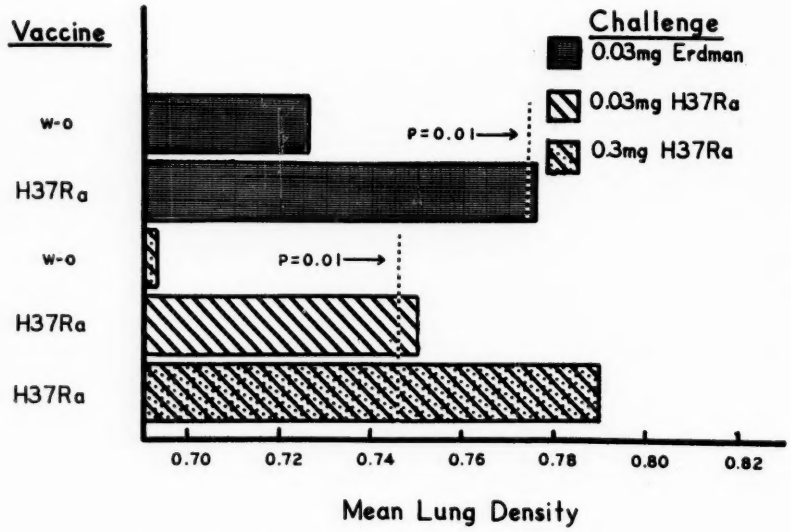

Fig. 2.—Mean lung densities of various groups of vaccinated (H37Ra) and control (w-o) mice challenged intravenously with living, virulent (Erdman) or avirulent (H37Ra) tubercle bacilli. Lung densities were determined forty-eight hours after challenge. Two different challenge doses of avirulent bacilli were employed. "*P*" indicates the statistical significance of the results.

effects of a common cause. To substantiate these points and to observe the effect of using a large challenge, an experiment employing five groups of seven mice each was constituted as follows:

Group 1: Vaccinated with control emulsion and challenged with 0.03 mg. Erdman bacilli.

Group 2: Vaccinated with H37Ra emulsion and challenged with 0.03 mg. Erdman bacilli.

Group 3: Vaccinated with control emulsion and challenged with 0.3 mg. H37Ra bacilli.

Group 4: Vaccinated with H37Ra emulsion and challenged with 0.03 mg. H37Ra bacilli.

Group 5: Vaccinated with H37Ra emulsion and challenged with 0.3 mg. H37Ra bacilli.

Two weekly intraperitoneal injections were given; each consisted of 0.1 ml. of w-o emulsion, with or without living H37Ra bacilli. The bacillary dose per injection was 0.5 mg. The mice were challenged two weeks after the second vaccination and forty-eight hours later their lung densities were determined.

As the results in Fig. 2 show, both virulent and avirulent human tubercle bacilli were equally capable of eliciting characteristic delayed lung reactions in mice vaccinated with the avirulent strain; these reactions are significant statistically. A tenfold challenge dose increase did not appear to aggravate the reaction proportionately. As in the first experiment, overt systemic tuberculin shock and anaphylactoid reaction were absent. Again, lungs from sensitized mice were found congested and hyperemic.

Despite informed opinion that vaccination with living, avirulent tubercle bacilli suspended in w-o emulsion should be a highly effective sensitizing technique in most animal species,[5] the classic method of inducing low-grade tuberculous infection to sensitize might be more effective in mice. It has been the method most commonly used by other experimenters dealing with this problem (see discussion). The fact that mice strongly resist subcutaneous tuberculous infection[6] suggested the practicality of an experiment containing three groups of ten mice each.

Group 1: Challenged intravenously with 0.03 mg. living Erdman bacilli suspended in 0.2 ml. buffer forty-eight hours before autopsy and twenty-one days after beginning of experiment.

Group 2: Vaccinated subcutaneously with 0.03 mg. living Erdman bacilli in 0.1 ml. buffer twenty-three days before autopsy; not subsequently challenged.

Group 3: Vaccinated as in Group 2 and challenged twenty-one days later, as in Group 1.

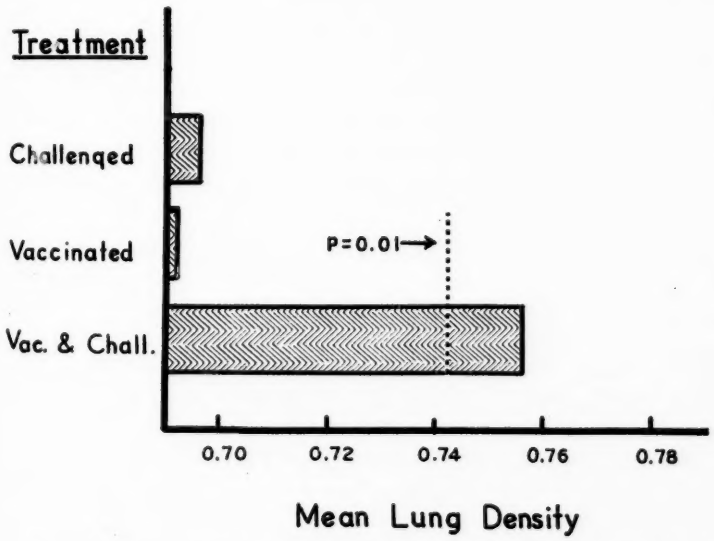

Fig. 3.—Mean lung densities of mice (1) challenged intravenously with 0.03 mg. living virulent tubercle bacilli, (2) vaccinated subcutaneously with 0.03 mg. living virulent tubercle bacilli, or (3) vaccinated subcutaneously with 0.03 mg. and then challenged intravenously with 0.03 mg. living virulent tubercle bacilli. Lung densities were determined at the same time for all three groups forty-eight hours after challenge of the upper and lower groups. "P" indicates the statistical significance of the results.

Fig. 3 shows the mean lung densities of the three groups determined at the same time in the experiment and forty-eight hours after challenge of Groups 1 and 3. Neither subcutaneous infection twenty-three days before autopsy nor intravenous infection forty-eight hours before autopsy increased mouse lung densities. Since these were the vaccinating and challenging procedures, respectively, it is obvious that the statistically significant mean lung density increase of the third mouse group is the specific consequence of challenge following vaccination. The severity of reaction was no greater than that seen in the previous two experiments. If this is an indication of the degree of hypersensitivity induced, then low-grade infection with virulent tubercle bacilli does not induce greater reactivity, under the conditions employed in these experiments, than vaccination with w-o emulsified avirulent tubercle bacilli.

The delayed lung reaction which has been the subject of these experiments appears to be specific and classifiable as an immunologic phenomenon. Evidence suggests that it is a manifestation of delayed hypersensitivity. Direct proof of this might be obtained by transferring this hypersensitivity passively from sensitized to unsensitized mice using the types of cells thought capable of this kind of transfer in other animal species but failing to do so using serum from sensitized donors alone. Preliminary attempts at cellular passive transfer with mice so far have not been successful in this laboratory. Evidence has been gathered, however, to show that the delayed lung reaction cannot be due to an immediate (anaphylactic) type of hypersensitivity.

Perhaps the most pointed reason for suspecting immediate hypersensitivity as the cause of this reaction, aside from current common knowledge that it is not difficult to induce in mice, is the fact that entire tubercle bacilli were employed as challenging antigens and that as coherent structures they could effect this reaction by (1) depositing in the lung and (2) releasing eliciting antigen only slowly into surrounding tissues. Bacilli have been employed in these experiments because they were being used when the reaction first was observed and because they seem better than tuberculin or tuberculoprotein alone for detecting delayed hypersensitivity.[7, 8] Two overlapping approaches were made toward showing that neither a general immediate hypersensitivity reaction with delayed consequences nor an artificially delayed and localized hypersensitivity reaction was responsible for delayed lung reactions in vaccinated and challenged mice. The first approach employed active induction of immediate hypersensitivity to bovine gamma globulin (BGG) and determination of lung densities during and after anaphylaxis; the second showed that passively transferred immediate hypersensitivity to tuberculoprotein did not predispose to the delayed lung reaction following intravenous injection of either the protein or entire tubercle bacilli.

Two BGG experiments were set up. In the first, a group of mice was sensitized to BGG with two simultaneous subcutaneous injections in opposite groins of 0.1 ml. w-o emulsion containing, between them, 0.1 mg. of tuberculolipid adjuvant and 0.1 mg. of BGG. Ten days later members of this and of a second control group, vaccinated solely with emulsion, were challenged intravenously with 0.05 mg. BGG in 0.1 ml. phosphate buffer.

Whereas the control mice were unaffected by this challenge, all the test mice developed severe anaphylaxis within five to fifteen minutes, this reaction reaching a climax about thirty minutes after challenge. The mean lung densities of both groups determined at this time, during acute anaphylaxis, were essentially normal, being 0.71 and 0.67 for test and control groups, respectively. No hemorrhagic congestion was apparent; the color in both sets of lungs was normal.

In mice of this experiment combination of antigen with antibody probably was quite rapid. Since such rapid combination may not have favored manifestation of a delayed reaction, in the next experiment mice sensitized with BGG as in the first, but with H37Ra tubercle bacilli employed as adjuvant, were challenged along with unsensitized control mice with three succeeding BGG injections by different routes. This challenge procedure was calculated to achieve desensitization followed by little or no deviation of antigen from potential sites of the hypothetical delayed reaction. Such a method of challenge might have the same results as slow diffusion of antigen from tubercle bacillary bodies postulated as a possible occurrence in the previous experiments where delayed reactions were seen.

TABLE I. SUMMARY OF THREE IMMEDIATE HYPERSENSITIVITY EXPERIMENTS

EXPERIMENT	SENSITIZATION	INDUCTION PERIOD	CHALLENGE	ROUTE*	AUTOPSY: TIME AFTER CHALLENGE	MEAN LUNG DENSITY	
						TEST	CONTROL
First BGG	Active	10 days	0.05 mg. BGG	I.V.	30 minutes	0.71	0.67
Second BGG	Active	24 days	0.1 mg. BGG	S.C.	= 48 hours	0.70	0.72
			1.0 mg. BGG	I.P.			
			1.0 mg. BGG	I.V.			
Tubercle bacillus and protein	Active	10 days	1.0 mg. protein	I.V.	48 hours	0.70	
	Passive	24 hours	1.0 mg. protein	I.V.	48 hours	0.68	
	Passive	24 hours	0.03 mg. bacilli	I.V.	48 hours	0.69	
	Passive	48 hours	0.03 mg. bacilli	I.V.	48 hours	0.69	

*I.V. = Intravenous.
I.P. = Intraperitoneal.
S.C. = Subcutaneous.

After the presence of anaphylactic hypersensitivity had been proved by preliminary challenge of a portion of the vaccinated mice, the rest and their unsensitized controls were challenged subcutaneously with 0.1 mg. BGG. This was sustained without obvious deleterious effect by both test and control mice. Twenty-four hours later members of both groups were injected intraperitoneally with 1 mg. BGG, and when this treatment also had no effect they received a third injection, one hour later, of 1 mg. BGG by the intravenous route. This final challenge, which was fatal to two of five of the same mouse group used without desensitization to prove anaphylactic sensitivity present and nearly fatal to the other three, caused no illness. Lung densities of these mice, taken forty-eight hours after the first (subcutaneous) challenge, were practically the same and nearly normal for test and control mice (Table I).

This kind of suggestive evidence that immediate hypersensitivity was not causing delayed lung reactions upon challenge was substantiated by the following experiment employing tubercle bacilli and tuberculoprotein. Twenty-five mice were vaccinated with two simultaneous subcutaneous injections, just as in the first BGG experiment, except that tuberculoprotein replaced BGG as antigen. Ten days later five of these mice were challenged intravenously with 1 mg. of tuberculoprotein. Four of the five rapidly developed severe anaphylactic shock, while the fifth showed a moderate degree of shock. All five recovered, however, and when they were autopsied two days later they exhibited normal lung densities. The remaining twenty sensitized mice were bled to death, the bloods were pooled and allowed to clot, and the resulting serum was employed for passive sensitization of a second group of twenty-five previously untreated mice.

Each recipient was injected intraperitoneally with 0.22 ml. of undiluted donor serum twenty-four and forty-eight hours before challenge, these transfers being arranged so that all mice could be challenged at the same time with the same bacillary preparation. Twenty-four hours after passive transfer, five of these mice were challenged intravenously with 1 mg. tuberculoprotein to ascertain whether passive transfer of immediate hypersensitivity had succeeded. Three of these five quickly developed strong anaphylaxis, one developed intermediate anaphylaxis, and one developed very light anaphylaxis. Since all recovered, their lung densities also were determined forty-eight hours after challenge and, as in the actively sensitized mice, were found to be normal.

Inasmuch as the remaining twenty recipients now could be considered passively sensitized to tuberculoprotein (immediate hypersensitivity), each was challenged intravenously with 0.03 mg. of H37Ra, ten twenty-four hours after passive transfer and ten forty-eight hours after transfer. Table I, which summarizes results from this and the two preceding experiments, shows that the mean lung densities of both groups were normal when determined forty-eight hours after challenge. Immediate hypersensitivity, then, does not account for delayed lung reactions detected in vaccinated mice subsequently challenged intravenously with tubercle bacilli.

DISCUSSION

Debate as to whether or not mice can develop delayed (tuberculin) hypersensitivity resembles that, now receding, concerning their competence to show anaphylactic hypersensitivity. Much evidence used in both arguments has been negative; the phenomena were thought absent because they were not detected. Just as increasingly efficient induction and detection techniques have been used to prove mice anaphylactically sensitizable, however, improved methods seem likely to verify tuberculin sensitization. Unfortunately, advances along this line of experimentation seem to have been hampered by inadequately disseminated information, due largely to the fact that most work on delayed hypersensitivity in mice or rats has been done as an accessory to tuberculosis research, thereby attracting only limited attention from researchers in other fields.

Therefore, at a time when generally held ideas on the subject are in flux, I hope that the following short review of significant experiments may stimulate wider thought and experimentation in this virtually unexplored and in many ways seemingly peculiar field, particularly with nontubercle bacillus antigens. Because available data from experiments with mice and rats appear interchangeable relative to the subject of this short review,[6, 9-11] they will be used to supplement each other.

Although delayed hypersensitivity can be detected simply in guinea pigs, rabbits, man, and several other animal species by their reaction to tuberculin injected intradermally, this classic skin test never has been duplicated in mice or rats, even when they were proved sensitive by other techniques.[3, 4, 7, 10] Although, with some reason, this has evoked assumptions that mice and rats do not develop delayed hypersensitivity, it is worth while to recall that conditions can be arranged under which the skin of a guinea pig with delayed hypersensitivity to dinitrochlorobenzene, for example, becomes unreactive while the guinea pig itself retains normal reactivity.[12] Therefore, although a typical positive skin test would indicate the presence of tuberculin allergy, its absence has little significance other than to suggest that sensitized mouse and rat skin reactivity is not like that of guinea pigs. For the present, then, evidence from other kinds of tests and other criteria must be considered in deciding whether or not mice and rats can develop tuberculin allergy.

Smith and Hendrick[13] were among the first to induce and detect tuberculin shock in rats. In their studies of nutritional change effects on experimental tuberculosis in albino rats, they observed that when these animals had been infected intraperitoneally with 5 mg. of virulent human tubercle bacilli and were challenged by the same route between one and five months later with 0.5 ml. of commercial Old Tuberculin, they underwent systemic tuberculin shock. Some died, and those that recovered needed one to two days in which to do so. Interestingly, Vitamin A deficiency appeared to aggravate tuberculin shock, and adequate supplies of the vitamin seemed to diminish it.

Hehre and Freund[10] skin-tested tuberculous rats with tuberculin at various intervals after intravenous infection with virulent bovine tubercle bacilli. Although antigen absorbed from the injection site provoked severe and occasionally fatal shock in some of these animals, invariably it had no effect on the skin. They were able to elicit tuberculin shock in similarly infected rats by intraperitoneal challenge and inferred from this that changing challenge routes did not influence the systemic effect of tuberculin. The amount of tuberculin they used to induce fatal shock reproducibly was large (100 to 1,000 mg.) but not harmful to uninfected animals; however, smaller amounts (20 mg.) were employed with somewhat less uniform results.

Gerstl and Thomas[14] have presented analogous data for mice. Skin tests with 0.1 ml. of 0.1 per cent purified tuberculoprotein derivative in mice vaccinated intensively with living bovine or human tubercle bacilli were negative, whereas intrapleural or intratesticular tests in similarly vaccinated mice appeared to have some allergic effects. Thus, while control mice were unaffected

by either of these types of challenge, test mice developed testicular hemorrhages and tubule necrosis eighteen to twenty-four hours after challenge by that route, and mice challenged intrapleurally showed increased diffusion of lymphocytes and monocytes into the pleural cavity.

Selbie and O'Grady[15] noticed that mice superinfected intramuscularly developed temporary swelling of the superinfected leg which reached its maximum twenty-four hours after challenge and then receded. Primary infection, by comparison, caused transitory swelling as a consequence of injection trauma, and this was less intense and disappeared far sooner than the swelling of superinfection. The delayed-swelling reaction regressed before subsequent inflammation due to actual infection made its appearance. Time relationships suggested to these workers that the temporary, delayed superinfection swelling reaction that they observed was a delayed hypersensitivity reaction. Skin tests on these mice, with either tuberculin or tubercle bacilli, uniformly were negative. More recent experiments published by O'Grady[7] bear out these authors' interpretation. O'Grady describes apparent desensitization of tuberile bacilli-infected mice by repeated intramuscular Old Tuberculin injections, so that mice reacting quite obviously by delayed thigh swelling at the beginning of treatment showed only immediate transitory swelling characteristic of unsensitized mice after this course of treatment. It is instructive to note that mice infected with the larger of two test bacillary doses would not react to the muscle test until after they had been so tested twice, even though they could be shown hypersensitive by systemic tuberculin shock, or to entire tubercle bacilli injected into the muscle. Commenting on this, O'Grady points out that the ease with which tuberculin reactions can be brought about in mice depends both upon methods used and upon tissues challenged and, hence, it appears that persistently negative skin tests in these animals are no proof that the animals are not allergic to tuberculin.

As the discussion of tuberculosis pathology below will show, various mouse and rat strains probably differ remarkably in their capabilities to develop tuberculin allergy. This may explain the noteworthy success of the Gray and Jennings[4] footpad test, a close relative of the skin test. Whereas most laboratories have employed white mice for obvious reasons of convenience, these workers have made use of the black C57 strain of mice. The generally lower resistance to tuberculous pathologic changes exhibited by this and other pigmented mouse strains[6, 16] could be due to their becoming sensitized to tubercle bacillary constituents more readily than white mice. Striking differences even among white mice should not be unexpected, for they have been observed with regard to a very sensitive anaphylactic hypersensitivity test.[17]

Gray and Jennings[4] observed that tuberculin injected into the footpad of an allergic tuberculous C57 mouse caused the foot to swell a few hours after challenge and to remain swollen for at least forty-eight hours, after which foot size slowly returned to normal. Anaphylactic sensitivity apparently played no role in this reaction, and the feet of normal mice challenged identically did

not swell appreciably. Recently, Gray and Affleck[18] utilized the footpad technique for an interesting study of succeeding states of tuberculin anergy and allergy in slowly advancing mouse tuberculosis. This study suggests two often unrecognized reasons for mice and rats to develop no delayed hypersensitivity: (1) these animals may die of acute tuberculosis without having sufficient time to develop delayed hypersensitivity or its pathologic manifestations, or (2) they may be so severely infested as to remain anergic by a process where desensitization exceeds sensitization. Such difficulties as these might be minimized if avirulent or killed tubercle bacilli were used to induce allergy.

Demonstrating delayed hypersensitivity by the footpad test may be possible only in certain mouse strains. For example, Gastinel, Vaisman, and Dunoyer[3] could induce severe specific tuberculin shock in chronically infected tuberculous white mice by injecting them subcutaneously with tuberculin but detected no reactions in these mice either to skin tests or to the Gray and Jennings footpad test. Selbie and O'Grady[15] mentioned that their Strong A white mice which reacted to tuberculin injected intramuscularly did not react to tuberculin injected into the footpad.

The work of Gastinel, Vaisman, and Dunoyer[3] just cited is informative with regard to my lung density experiments for its description of organs in mice affected by tuberculin shock. The viscera of these animals, suffering severe shock after subcutaneous challenge, were found to be congested and hemorrhagic, and particularly the lungs were affected. Although challenges employed in the present lung density studies were insufficient to provoke overt symptoms, hemorrhagic lung congestion was a consistent pathologic characteristic of challenged, sensitized mice, and it would account physically for the lung density increases interpreted as reflecting delayed hypersensitive reactions.

Individually, the evidence from any single series of the experiments just discussed should be moderately convincing proof that mice and rats develop delayed hypersensitivity. Collectively, their evidence seems conclusive. If, then, mice and rats should be assumed so capable, they might be expected to exhibit the pathology of tuberculosis seen in the higher animals reactive to skin tests and thought closely related to this kind of allergy. Yet, it is common knowledge among those who use these animals in tuberculosis research that generally the microscopic and macroscopic tissue changes in tuberculous mice and rats are less extensive and less varied than in guinea pigs and rabbits, for example. Ordinarily, the pathology of these larger animals is described in terms of emergence of epithelioid cells, of giant cells, lymphocyte infiltration, caseation, fibrosis, and cavitation[9, 19-22] whereas in mice and rats tuberculosis commonly evokes proliferative or necrotic-exudative lesions, macrophage rather than lymphocyte infiltration, and foam cells.[14, 23, 24] Nevertheless, review of published data shows that, while not common, the changes seen in easily sensitized animals also have been observed in mice or rats and therefore that the latter animals probably need not be denied capability for developing tuberculin allergy on this basis.

Epithelioid cells have been observed in mice by Gray and Jennings[4] and by Pierce and associates[16] and in rats by Hehre and Freund.[10] Smith and Hendrick[13] found giant cells, multinucleated cells, epithelioid cells, and lymphocyte infiltration in infected rats. Giant cells and cells of the Langhans type are exhibited in photographs of sections of tuberculous rat lung published by Hehre and Freund.[10] Although caseation is not common in mice and rats, it is not rare.[3, 6, 14, 23, 24]

Formation of cavities in animals seems to be related to development of delayed hypersensitivity, if not dependent upon it. The recent intriguing experiments of Yamamura and co-workers[25, 26] substantiate this, for they show that cavities can be induced to form in rabbits by appropriate immunologic procedures making specific use of tuberculin allergy. Cavitation in tuberculous mice apparently has been observed in only one laboratory. Levaditi, Vaisman, and Barrat[27] found 20 per cent of one series of chronically infected mice sacrificed 139 days after infection to have developed cavities in the pulmonary parenchyma. A later experiment involving three batches of sixty mice was less successful in this respect, since only two mice developed cavities. Observed microscopically, these cavities showed encapsulation by fibroblasts, large monocytes, and lymphocytes and had attained diameters of 1 to 2 mm. They were surrounded by heavy concentrations of tubercle bacilli. Levaditi and co-workers believe that the cavities were formed by a progressive softening of large tuberculous follicles followed by pus evacuation through bronchioles, and they provide photographic evidence to substantiate this belief. Apparently, since such cavities could not be induced at will in subsequent mouse experiments, the conditions under which they can develop in mice must be rather specific and, at present, are not well enough understood to control. Perhaps from the stimulus which this discovery may provide, vigorous attempts to induce cavitation in these animals and to learn under what particular set of conditions it can develop will be made in the future.

Although neither direct nor indirect evidence that mice and rats can develop delayed hypersensitivity is available in large quantities, enough has accumulated to be nearly conclusive. Special techniques have had to be developed to gather it, since sensitized mice and rats have responded to the traditional skin test as the higher animals do. Perhaps the passive transfer experiments of Wallace[28] offer an explanation for the ineffectiveness of skin tests in rats. Since Wallace was able to transfer delayed hypersensitivity from rats to guinea pigs, using rat leukocytes, it would seem that some reactive property of guinea pig skin is lacking in rat skin.

The original experiments reported in this article offer further direct evidence by employng a sensitive new technique. They have indicated that mice need not be infected with virulent tubercle bacilli to develop easily detectable sensitization, that sensitivity can be observed after intravenous injection of only a few hundredths of a milligram of tubercle bacilli, and that demonstrable active or passive immediate hypersensitivity is not associated with the delayed reactions observed.

SUMMARY

A new technique has been used to show that white mice can develop delayed hypersensitivity to tubercle bacilli. The technique consists of determining mouse lung densities after intravenous challenge with these bacilli, increased densities indicating the delayed allergic reaction. Aside from its high sensitivity, this method takes advantage of the observation that in sensitized mice undergoing such a reaction the major organs primarily affected are the lungs.

The reported experiments show that living, avirulent tubercle bacilli suspended in water-oil emulsions sensitize as effectively as similarly injected but buffer-suspended living, virulent bacilli. The delayed hypersensitivity reaction could be elicited with as little as 0.03 mg. of either virulent or avirulent bacilli. Active and passive sensitization experiments showed that the observed delayed lung reaction was not due to an anaphylactic (immediate) type of hypersensitivity to tuberculoprotein.

A short review of the literature concerned with the question of whether or not mice and rats can develop delayed hypersensitivity is presented, and from this the conclusion is reached that available evidence overwhelmingly favors the competence of these species to become allergic.

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THE PROGNOSIS OF ASTHMA IN INFANCY

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IT IS remarkable that so little has been written about asthma in infancy. In the textbooks it is usually mentioned very briefly, if at all, and yet this condition is common and important.

It is common. In 1930 Bray,[1] using his own figures and other reported series, found that of cases of asthma in the first ten years of life, 23 per cent started in the first year and 17 per cent in the second year; thus 40 per cent of these patients started to wheeze before the age of 2 years. Any pediatrician taking histories from the mothers of patients finds that this early incidence of asthma is frequent.

It is important, for nearly half of the patients develop serious difficulty, and about one-fourth have long-continued handicaps of either moderate or severe asthma. In my practice the greater number of difficult and so-called intractable cases had their beginnings in infancy.

These remarks on the prognosis of asthma in infancy are based on the study of the records of eighty-seven asthma patients seen before the age of 2, and followed in the office or by letter for more than two years. The oldest is now 36 years of age, and the youngest is 3½ years; the average age is 9 years. The records not used in the study were those of patients that I saw only once and those from whom I was unable to get a report two years later. The reports are not an ideal cross section of asthma in infancy; my earlier cases were indexed as asthma only when they were fairly severe, and thus there are a few more severe cases than there should be in a fair sampling.

It should be noted that many of these patients were treated for long periods to the best of my ability, some had lesser degrees of treatment, and some had no treatment worthy of the name. All of these are included in the figures.

Of the eighty-seven patients, forty-eight, or 55 per cent, have done no wheezing at all during the last year of recorded progress or follow-up. Thirty-seven, or 43 per cent, have done at least some wheezing during the last year. Two patients have died.

One of the patients who died was 10 months old. I had seen him five times, and he seemed to be doing very well. Then it was reported to me that he developed a respiratory infection, a temperature of 107 degrees, and a white count of 35,000 and died after being sick two days. This death would seem to be due to an overwhelming infection not related to the asthma. The other patient who died lived to be 7 years old. I saw her nine times during her second and third years. She was wheezing a little every night, but her

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general condition was good and my skin tests were all negative. She was treated for three years in another city, finally became worse, and died in Arizona "from asthma," according to a letter from the mother. It would seem possible that in this case there might have been present some other condition which caused her death, maybe a congenital abnormality of the trachea or bronchus.

In general, the mortality among asthmatic children is small. Rackemann and Edwards[2] found that 2.4 per cent had died after twenty years, but this includes some years of adult life, and only 0.8 per cent died of asthma. During the last few years I have heard several very competent observers express the opinion that the mortality is increasing with the increased use of cortisone and related compounds. Probably it is wise in treating asthmatic children to avoid these as much as possible, except very occasionally for short periods in acute conditions.

Of the whole group, 55 per cent were completely well for at least one year at the time of the last report. Dr. Rackemann reports a series of children after twenty years; nearly three-fourths were cleared of asthma in their teens. The cases first seen in infancy that I am reporting at present have not yet done as well as Dr. Rackemann's children of all ages. As the average age of the children in my series was 9 years at the last report, it is probable that after another five or six years the results will be more encouraging. Maybe the ultimate outlook for infants with asthma is as good as for children who develop asthma later.

More interesting and instructive is the course of the asthma in certain groups of babies. Nineteen of the infants had asthma, definitely positive skin tests for egg, and atopic dermatitis. In this group the prognosis for early recovery was very bad. Sixteen of the nineteen are still wheezing, at least to the extent of having wheezed some during the last year; two, after a prolonged illness, are now not wheezing; and only one had a fairly good course with recovery at eleven years. The bad prognosis in these cases can be brought out by reviewing briefly the histories of the only three who were not wheezing at the last report.

M. M. was seen in his second year. He had had asthma since the age of 7 months. He had attacks of wheezing, a history of atopic dermatitis, many large positive skin test reactions (including egg), and many clinical allergies. I treated him for ten years. The only place where he was fairly well was the Lakeside Preventorium, and here he wheezed a litle most of the time. When he was 11 years old the family moved to Arizona, and since then he has steadily improved. At the age of 18 he reports that he no longer wheezes.

J. M. was seen with asthma and moderate atopic dermatitis in his second year. His skin tests showed large positive reactions to egg and other foods. I treated him for twelve years. At the age of 14 he was wheezing only a little bit occasionally, and at 15 years of age he stopped wheezing.

D. D. was seen by me at 11 months of age, with occasional wheezing, a mild atopic dermatitis, and several positive skin test reactions, including a large one to egg. Now, at 12 years of age, he has had no wheezing for the past one year.

The first two of these patients had severe asthma which cleared up in their teens. The third patient had a mild asthma which last appeared at the age of 11. These three cases would lead me to hope that, in time, other patients who are now younger will be well. It is evident, however, that infants with asthma, atopic dermatitis, and positive skin test reactions to egg are likely to have severe asthma and are likely to have a serious time with it.

There were twelve asthma patients who had or had had what was believed to be atopic dermatitis but who had negative skin test reactions to egg. Of these, seven are well and five are still wheezing. These small figures may suggest that eczema without positive egg tests is not of such bad prognostic import.

There were thirty patients who had entirely negative skin tests. Of these, twenty-one were not wheezing at the last report, and only nine still had symptoms. Apparently, the prognosis is much better when all the tests are negative.

All this would seem to indicate that the capacity to develop hypersensitiveness varies in degree. According to this supposition, the patients with the three manifestations of allergy developing at an early age have the greatest capacity to develop hypersensitivity. Therefore, they have the greatest degree of sensitivity, the greatest number of sensitivities, and the most difficult time. The asthmatic infants who have negative tests and no dermatitis have the least degree of sensitivity and the fewest sensitizations, and thus they usually have the easiest time and the best outlook. Although there are exceptions in both groups, this conclusion would seem to be valid.

Of these egg-positive patients, the youngest at the first testing was 4 months old; he gave an enormous positive test reaction to egg at this time. This positive egg test in young infants would seem to be an example of what Ratner[3] called congenital allergy, caused by active sensitization in utero, and also this would seem to be a sign of a great capacity to develop sensitization. Hill,[4] in 1949, made a statement along these lines. He said, "The egg white reaction in infants may be looked upon as an index of allergy rather than a help in determining the cause of symptoms."

There were twenty-three patients with positive egg tests at the first testing, and I was able to retest sixteen of these up to the average age of 7 years. At this time, one-half of them still had a positive test reaction to egg and one-half had a negative reaction. Thus, the positive egg test, which is such a valuable sign in infancy, does not necessarily persist, although it may persist and continue to be a valuable sign.

Forty-eight of the eighty-seven babies were retested at a later period. In no case was a positive egg test found where it had previously been negative; in none of these cases was a positive egg test known to develop after the first testing. This is an interesting observation, but it may not be confirmed when more cases are studied.

It has been frequently stated that it is not necessary to skin-test a baby with asthma. There are many arguments that might be used against this delay

in skin testing, and one of these is pertinent to the present discussion. The skin testing tells us a great deal about the probable course of the disease—whether prompt improvement may be expected or whether we are in for a long, hard struggle. This makes a difference in the expense involved in preparing the patient's house and even in the place of residence. It bears on the problem of how much trouble should be taken in planning dietary experiments and on the question of air conditioning. Above all, letting the patient's mother realize that she is in for a long, hard struggle saves her from the demoralizing effects of repeated disappointments.

SUMMARY

1. Asthma in infancy is common and important.

2. A follow-up of eighty-seven patients seen with asthma before the age of 2 years (now at an average age of 9 years) shows that forty-eight, or 55 per cent, have not wheezed in the last year.

3. Of the nineteen patients with asthma, atopic dermatitis, and a positive skin test reaction to egg, only three have not wheezed during the last year.

4. Of the thirty patients with negative tests at the first testing and with no atopic dermatitis, twenty-one have had no wheezing this year.

5. It would seem that the capacity to develop sensitiveness varies in degree, that the patients with the greatest such capacity have the poorest prognosis, and that generally this capacity can be roughly estimated at the first visit.

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RESERPINE EFFECTS AND EXPERIMENTALLY PRODUCED LESIONS OF CARDIOVASCULAR SENSITIZATION AND EOSINOPHILIA

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INTRODUCTION

THE physiologic mechanisms or circumstances associated with hypersensitivity phenomena are not as clearly defined as the well-documented serologic changes and histologic responses noted in such reactions. Although the action of histamine[1-3] participation may offer an explanation for several of the manifestations of anaphylaxis, there is still insufficient evidence for its role to be accepted as the sole agent in the experimental animal. Recent reports suggest that serotonin released in a physiologically active state may be etiologically implicated in rabbit anaphylaxis[4-6] and related immunologic reactions.[7-9] In further exploration of such a possibility, an attempt was made to study relationships between serotonin release and some of the histologic manifestations associated with hypersensitivity reactions, including experimentally produced lesions of the cardiovascular system[10-11] and tissue eosinophilia.[12] Advantage has been taken of the well-defined actions of reserpine in releasing and depleting[13-15] body stores of serotonin. Rabbits treated with reserpine in an attempt to achieve such serotonin modification were then subjected to experimental procedures affecting tissue-sensitizing mechanisms.

EXPERIMENTAL PROCEDURES AND RESULTS

Cardiovascular Lesions

Materials and Methods.—Thirty male rabbits, each weighing between 2 and 3 kilograms, were subjected to active sensitization and challenge procedures. Each animal received a total of 10 ml. of sterile bovine serum absorbed on aluminum hydroxide gel in divided doses by intramuscular and subcutaneous injections given at multiple sites on the same day. Seventeen to nineteen days later, when it was ascertained by interfacial serum precipitin tests that circulating antibodies to the bovine serum proteins were present, each rabbit was subjected to a subanaphylactic challenge with homologo-specific antigen. Bovine serum diluted 1:100 in physiologic saline was administered by slow intravenous drip into an ear vein at the rate of approximately 20 to 30 drops per minute until a maximum total of 100 ml. of this dilution had been reached.

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If the animal showed any signs of impending anaphylaxis, such as sneezing or sniffling, excess salivation, nasal discharge, restlessness, or labored breathing, the challenging injection was immediately stopped until all such symptoms had subsided. In this manner, the time interval during which the entire dose of challenging antigen was administered often varied from two to five hours. In an occasional instance the development of recurrent symptoms upon reinstitution of the intravenously administered antigen prevented the administration of more than a total of 30 to 50 ml. of the bovine serum dilution. Twenty-four hours after completion of the challenging injections the rabbits were sacrificed by air embolism, and multiple sections taken from the lungs, heart, pulmonary artery, aorta, and kidneys of each animal were prepared for microscopic study.

Fifteen of these sensitized and challenged rabbits were also treated with reserpine. Beginning on the day of initial administration of the bovine serum, and daily thereafter, each rabbit in this group received a subcutaneous injection of reserpine* 0.1 mg. per kilogram of body weight. Fifteen minutes before the challenging injection of bovine serum this same dosage of reserpine was given by the intravenous route.

Histologic Findings.—The following types of microscopic lesions arising on the basis of hypersensitivity mechanisms were most consistently noted in this study:

Arteritis: The small pulmonary arteries and pulmonary arterioles were diffusely and segmentally involved in pathologic processes resulting in the histologic pictures of periarteritis and panarteritis (Fig. 1). Typical lesions exhibited marked perivascular and adventitial cellular infiltrations consisting chiefly of eosinophils, some polymorphonuclear leukocytes, and, to a lesser extent, lymphocytes. Eosinophils were found infiltrating the intima and media of these vessels, and in many instances collections of eosinophils were found in large clumps within their lumina. The intima was also involved with varying degrees of hyperplasia of endothelial cells. In addition to cellular infiltrations, there was associated edema of the adventitia and media, and occasionally swelling and disorganization of the medial fibers were noted. A second type of pulmonary vascular lesion less commonly demonstrated was of the granulomatous type (Fig. 1, *C*). These lesions were characterized by nodular periarteriolar cellular accumulations consisting chiefly of polymorphonuclear leukocytes with lesser numbers of eosinophils and a few lymphocytes and mononuclear cells. Some rabbits presented an equivocal degree of perivascular involvement of a few arterioles and venules. These were characterized by thin periadventitial ringing with few eosinophilic granular cells, and occasionally these were associated with adherence of a few eosinophils to the intimal surfaces but without any true

*Serpasil supplied through the courtesy of Dr. Albert J. Plummer, Director of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

changes in the intima or alterations of the media. Mild degrees of perivascular ringing with polymorphonuclear leukocytes involving coronary vessels and vasa vasorum at the base of the aorta were occasionally observed.

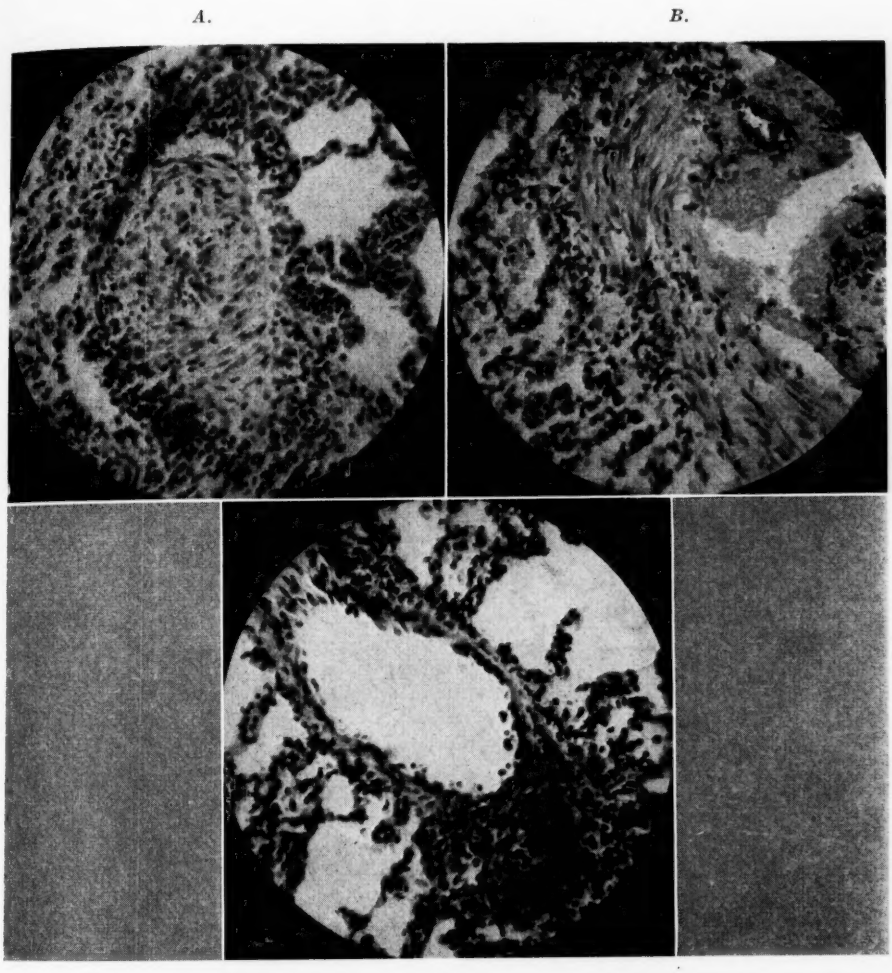


Fig. 1.—Sections of lungs from rabbits actively sensitized to bovine serum and challenged with subanaphylactic doses of homologous antigen. These demonstrate typical lesions of arteritis. Periarterial and adventitial infiltrations with eosinophilic granular cells are evident and eosinophils can be seen penetrating the media in *A* and *B*. Intimal reaction is noted in *A* and thrombus formation in *B*. A granulomatous type of lesion consisting of polymorphonuclear leukocytes and a few eosinophils involving a pulmonary arteriole is seen in *C*.

Myocarditis: Myocardial involvement was demonstrated in varying degrees and was classified as follows: (1) marked (Fig. 2, *A*)—extensive and diffuse cellular infiltrations throughout sections of the myocardium consisting of polymorphonuclear leukocytes and eosinophilic granular cells, associated interstitial edema of the cardiac musculature, and occasional endocardial infiltrations with eosinophils,

and (2) mild—either nodular focal collections of eosinophilic granular cells (Fig. 2, *B*) or diffuse and light infiltrations of eosinophilic granular cells scattered throughout the myocardium.

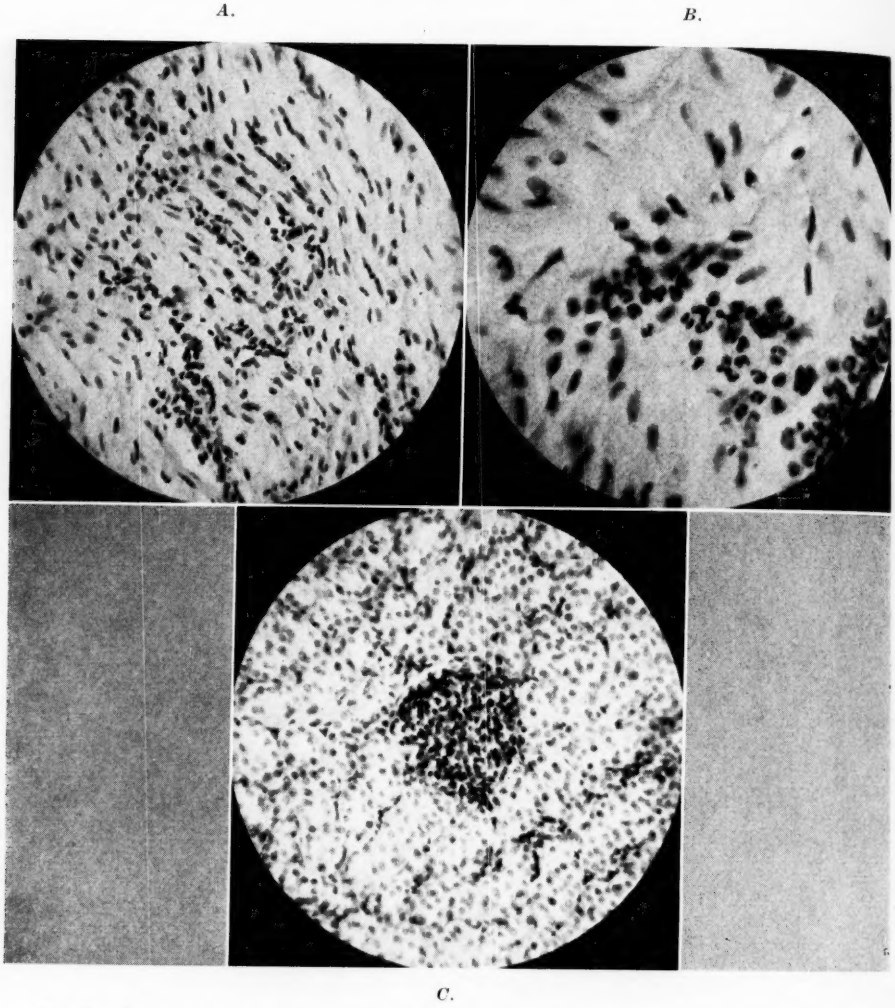


Fig. 2.—Sections of heart (*A* and *B*) and kidney (*C*) from rabbits that had been actively sensitized to bovine serum and challenged with subanaphylactic doses of homologous antigen. Diffuse myocardial cellular infiltration is seen in *A* and a focal nodular accumulation of eosinophilic granular cells in *B*. A lesion of proliferative glomerulitis from a rabbit that had been simultaneously treated with reserpine is seen in *C*.

Glomerulitis: The glomeruli were the only portions of the kidney which appeared to be involved, and these were affected in varying degrees and numbers. These affected glomeruli were somewhat swollen, and their pathologic alterations were chiefly of an infiltrative and proliferative nature (Fig. 2, *C*) resulting in increased glomerular cellularity. Increased numbers of cell nuclei were seen within glomerular tufts with occasional swelling of endothelial cells and

basement membranes. Cellular infiltrations within the capillaries included polymorphonuclear leukocytes, eosinophilic granular cells, and mononuclear cells.

Results.—In all thirty rabbits of both the control and reserpine-treated groups, serum-precipitating antibodies to beef serum proteins were demonstrated just prior to intravenous antigenic challenge.

The microscopic pathologic findings in the heart, lungs, and kidneys are summarized in Table I.

TABLE I

LESIONS	NUMBER OF RABBITS	
	CONTROL GROUP (15)	RESERPINE-TREATED (15)
Myocarditis	15	15
(a) Marked	6	0
(b) Mild	9	15
Proliferative glomerulitis	11	10
Pulmonary arteritis		
(a) Periarteritis and panarteritis	15	6
(b) Equivocal periarterial changes	0	4
(c) None	0	5

Experimental Eosinophilia

Materials and Methods.—The technique for inducing eosinophilia of the peripheral blood and bone marrow and eosinophilic granular cell infiltrations into cutaneous tissues, previously described,[16] was again employed in this study. Twelve male albino rabbits of the New Zealand strain, ranging in weight from 1.5 to 2.5 kilograms each, were used as experimental animals throughout the study. Crude beef-liver extract* in a strength of 13 mg. protein nitrogen per milliliter was employed as the eosinotactic antigen. Ten milliliters of liver extract mixed with 2 ml. of aluminum hydroxide gel was given to each animal by intramuscular injections at multiple sites on the first and eighth days of the experiment. In addition, seven spaced injections, each containing 1 ml. of liver extract, were administered through the ear veins of all animals during the first fourteen days.

Six rabbits received crude beef-liver extract alone. The remaining six were treated with reserpine in daily doses of 0.1 mg. per kilogram of body weight injected subcutaneously during the entire experimental period.

Total eosinophil counts were determined on all animals prior to the initial injections of beef-liver extract, and these were repeated at two- to three-day intervals throughout the duration of the experiment. Propylene glycol solution containing 0.1 per cent phloxine was employed in total eosinophil counts, according to the technique described by Pilot.[16]

On the sixteenth day animals in both groups received intracutaneous injections of 0.75 ml. of crude beef-liver extract plus 0.25 ml. aluminum

*Supplied by Armour Laboratories, Kankakee, Illinois, through the courtesy of L. W. Van Ness and by the Pitman-Moore Co., Division of Allied Laboratories, Indianapolis, Indiana, through the courtesy of Dr. Phillip A. Boyer, Jr.

hydroxide gel at a dorsolateral site midway between the pelvic and pectoral regions. Biopsy specimens of these locally injected skin sites were taken forty-eight hours later and prepared for microscopic study. To minimize confusion with the "psuedoeosinophils" commonly observed in the rabbit, all tissue cells were carefully studied in order that the characteristic eccentrically placed, bilobed, plump, round, blue-staining nucleus and the characteristic coarse, red, cytoplasmic granules might be identified.

Following the completion of injections of beef-liver extract, and of reserpine in those rabbits so treated, each animal was bled from the central artery of the ear and the serum was separated and tested for the presence of circulating precipitating antibodies to beef-liver extract by the ring test technique. The development of an antigen-antibody reaction was indicated by the formation of precipitate at the interfacial junction of the beef-liver extract dilution layered over the rabbit serum.

Following completion of the skin biopsy on the eighteenth day, the animals were sacrificed by air embolism, and marrow smears prepared from the femoral bone were treated with Wright-Giemsa stain for differential cell study.

Results.—

Peripheral blood eosinophils: A progressive rise in circulating eosinophils was noted in all members of the control and reserpine-treated groups of rabbits (Table II and Fig. 4). In those animals receiving only crude beef-liver

TABLE II. PERIPHERAL BLOOD TOTAL EOSINOPHIL COUNTS OF RABBITS TREATED WITH PARENTERAL CRUDE BEEF-LIVER EXTRACT

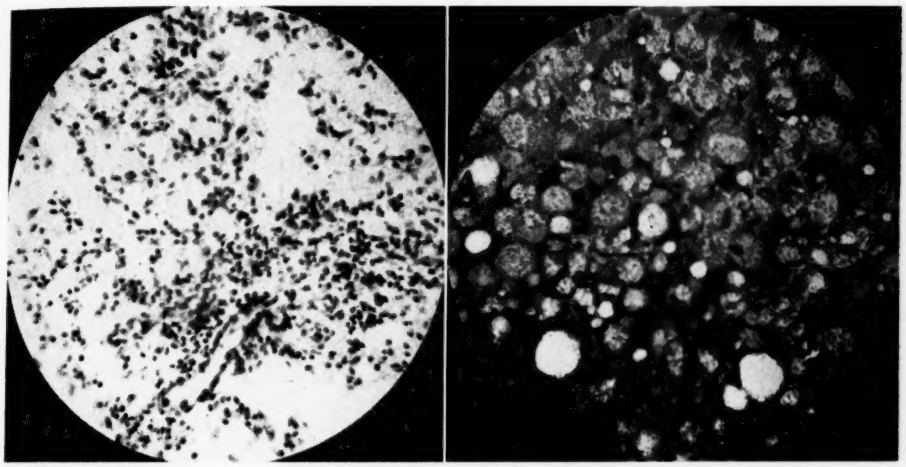
RABBIT NO.*	EOSINOPHILS PER C. MM. DAY NUMBER				
	1	4	8	12	16
1C	778	902	1452	1100	1188
2C	492	635	481	508	712
3C	759	1375	1837	1790	2131
4C	644	1199	1232	1482	1721
5C	555	1099	1132	1400	1687
6C	666	1010	1054	1277	1521
Mean	649	1037	1181	1258	1493
1S	1014	1724	1713	2167	1834
2S	943	547	585	1267	1559
3S	721	723	1256	1289	1009
4S	734	1223	2063	1427	1491
5S	567	743	1513	699	987
6S	699	1139	1397	1757	1287
Mean	663	1016	1421	1434	1361

*Rabbits 1S to 6S also received daily subcutaneous injections of reserpine.

extract the average eosinophil count rose from an initial level of 649 to 865 cells per cubic millimeter on the fourth day and gradually increased to a level of 1,493 cells per cubic millimeter at the termination of the experiment on the sixteenth day. A similar pattern was noted in those animals simultaneously treated with reserpine and beef-liver extract. Here the average initial eosinophil count of 663 per cubic millimeter rose to 1,016 on the fourth day and

to 1,434 on the twelfth day of treatment, with a final average count of 1,361 per cubic millimeter recorded at the termination of the experimental procedure.

Bone marrow smears: Differential cell study of the femoral bone marrow of all twelve rabbits in both the control and the reserpine-treated groups revealed similar pictures (Fig. 3, *B*). In each smear there were demonstrated a few mature lymphocytes, an increased number of myelocytes, an average number of polymorphonuclear neutrophilic leukocytes, and a very marked increase of eosinophilic granular cells. The marrow was found to be completely congested with intact mature, immature, and precursor eosinophilic granular leukocytes and evidence of extracellular, nonphagocytized, eosin-staining granules.



A. *B.*

Fig. 3.—Skin biopsy (*A*) and bone marrow smear (*B*) from rabbit actively sensitized with crude beef-liver extract. Infiltrations of eosinophilic granular cells with associated interstitial edema are seen in the corium of a biopsy section (*A*) from a cutaneous site injected with homologous antigen forty-eight hours previously. The bone marrow (*B*) is intensely congested with mature and immature eosinophilic granular cells and extracellular eosin-staining granules. Similar findings were also noted in rabbits simultaneously treated with reserpine.

Skin sections: Microscopic examinations of the cutaneous sites locally injected with liver extract and biopsied forty-eight hours later revealed similar histologic pictures for each of the twelve rabbits comprising both the control and the reserpine-treated groups (Fig. 3, *A*). Each section exhibited extensive localized infiltrations of eosinophilic granular cells into the epidermis and corium. Interstitial tissue edema was usually noted in association with these cellular infiltrations.

Antibody determinations: The development of precipitating antibodies to beef-liver extract was demonstrated in the sera of all rabbits completing the series of beef-liver injections, including those simultaneously treated with reserpine.

COMMENT

The experimental procedure employed in this study was designed to effect immunologic conditions optimum for the production of histologic manifestations of hypersensitivity reactions in animals depleted of serotonin.

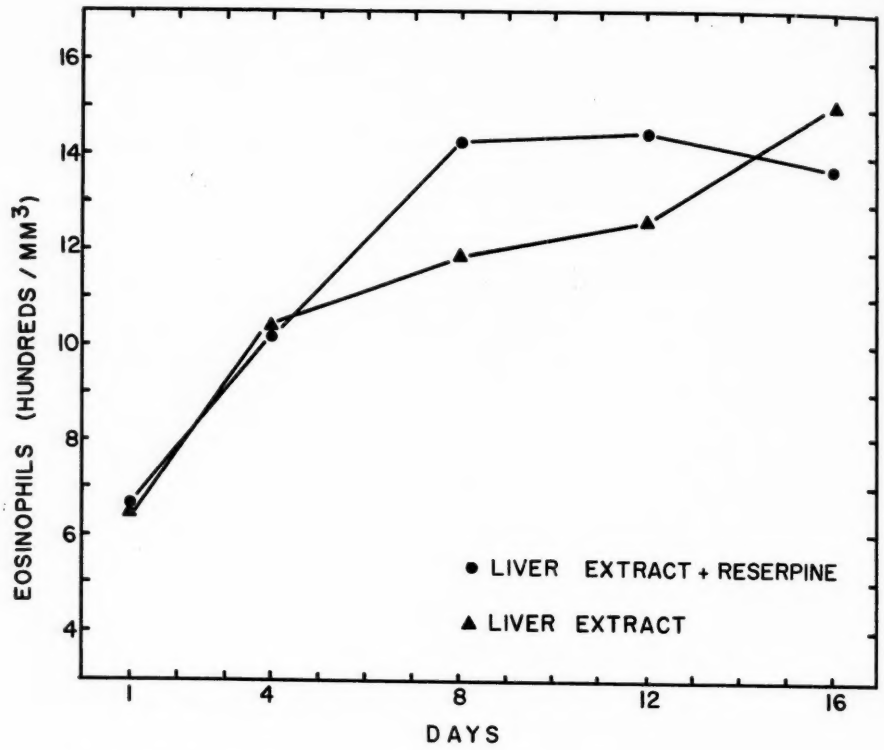


Fig. 4.—Graph showing mean of peripheral blood total eosinophil counts of those rabbits treated with liver extract alone and of those treated with liver extract and reserpine simultaneously.

The dose of 0.1 mg. per kilogram of reserpine[6] has been found capable of releasing and depleting platelets of serotonin content, although dosages up to 5 mg. per kilogram may be required for complete depletion of other serotonin-containing tissues, brain and intestine.[13] Although the manifestations of rabbit anaphylaxis have been shown to be related to participation of circulating blood platelets[17] and possibly to their specific serotonin content,[4-6] other results might have been evident at lower tissue levels of intestinal and brain serotonin. However, repeated daily dosages prerequisite for the production of such a state result in toxic levels of reserpine; this prevents the survival of rabbits over the required eighteen-day experimental period.

Among other variables in the evaluation of histopathologic modifications resulting from reserpine treatment, consideration should be directed to its associated histamine-,[6] epinephrine- and norepinephrine-[18]releasing effects.

It is, therefore, noteworthy in the evaluation of possible reserpine effects on experimentally produced hypersensitivity pulmonary arteritis that (1) although rabbit platelets contain both serotonin and histamine,[19] serotonin is also found in the lung[20] and (2) antihistaminic agents alone have been found generally ineffective in modifying vascular lesions of this type.[21-24]

Evident differences were noted in the action of reserpine at these dosages in influencing the development of experimentally produced lesions of the heart and pulmonary vasculature in contrast to those involving the glomeruli. Twenty-one of thirty animals subjected to sensitization and challenge techniques showed histologic evidence of the development of varying degrees of glomerulitis, with no significant differences in distribution noted between the control and reserpine-treated groups. On the other hand, although reserpine treatment failed to prevent the development of myocarditis, the predominance of very mild lesions was evident in rabbits so treated. Although the lesions of pulmonary periarteritis and panarteritis are difficult to quantitate because of their segmental nature of occurrence, a study in uniformity and qualitative degree of vascular involvement demonstrated an apparent attenuating effect of reserpine in this regard. The fact that typical acute vascular lesions could be demonstrated in less than one-half the number of reserpine-treated rabbits may be considered significant in view of the particular sensitizing and challenging technique employed. This resulted in the successful uniform production of vasculitis in all animals of the control group. An explanation for the differences in organ involvement is not apparent, but it may be concerned with observations that proliferative glomerulitis is the most consistent lesion of vascular hypersensitivity and its development as an early and sensitive indicator of the in vivo antigen-antibody reaction.[25-27] In this further regard, it is of interest that the antihistaminic agent, promethazine hydrochloride, demonstrated to be ineffective in modifying the development of experimentally induced serum hypersensitivity glomerulonephritis in the rabbit,[28] has recently been shown also to possess antiserotonin properties.[29]

Although the exact function of the eosinophilic leukocyte is not known, its association with in vivo reactions of hypersensitivity and allergic phenomena is a familiar and well-documented picture. It has been suggested that the eosinotactic stimulus is furnished by the release of an agent as a product of the antigen-antibody reaction or by one of the enzymes activated through such a reaction.[30] The possibility that this agent might be histamine is further suggested by reports indicating a possible role of eosinophilic granulocytes as carriers of histamine[31-32] or even associated with antihistaminic activity.[33] Since such a physiologic eosinotactic agent has not been definitely identified, it seemed worth while to study its possible association with serotonin activity through the serotonin-releasing and -depleting effects of reserpine. With dosages employed in this study, however, reserpine treatment failed to prevent or even to modify the increase in peripheral blood

eosinophils and their precursor cells in bone marrow or the infiltration of eosinophilic granular cells into cutaneous tissues provided by the antigenic stimulus of a foreign protein, such as crude beef-liver extract.

The results of these preliminary experiments pose several interesting and important questions relative to undetermined aspects of reserpine action and have directed our attention to the following further experimental approaches:

(1) Attempts to effect a more complete degree of serotonin tissue depletion and/or antiserotonin activity in the presence of hypersensitivity stimuli designed to produce histopathologic alterations over an experimental period of shorter duration. This would permit the administration of serotonin-modifying agents in maximum pharmacologic dosages without the interference of their cumulative untoward effects.

(2) Evaluation of the mechanism of attenuating effects of reserpine on experimentally produced hypersensitivity carditis and arteritis in relationship to releasing actions on tissue serotonin, histamine, and/or norepinephrine.

SUMMARY

1. A series of thirty rabbits were subjected to active sensitization and subanaphylactic challenge techniques with bovine serum favorable for the development of histologic lesions of glomerulitis, myocarditis, and pulmonary periarteritis and panarteritis. Fifteen of these animals were simultaneously treated with reserpine in an attempt to induce and maintain an associated state of serotonin tissue release and depletion.

2. The development of lesions of proliferative glomerulitis was found to be unaffected by reserpine treatment.

3. Evidence of myocarditis was found in all thirty animals, but it was considerably less extensive and of a milder degree in the reserpine-treated group.

4. All fifteen rabbits in the control sensitized and challenged group demonstrated lesions of pulmonary periarteritis and panarteritis. In five of the fifteen reserpine-treated animals evidence of vasculitis was absent and in the remaining four rabbits the lesions of pulmonary arteritis were of an equivocal nature, in contrast to those observed in the untreated group.

5. Crude beef-liver extract was administered to twelve rabbits in accordance with a technique designed to effect eosinophilia of the peripheral blood and bone marrow and eosinophilic granulocytic cellular infiltration into cutaneous tissue sites. Six of these animals were simultaneously subjected to the serotonin-releasing effects of reserpine.

6. Reserpine treatment failed to influence the development of peripheral blood or bone marrow eosinophilia or the histologic lesions of eosinophilic granular cell infiltrations into cutaneous sites.

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PENICILLINASE IN THE TREATMENT OF ALLERGIC REACTIONS TO PENICILLIN

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PENICILLINASE, the protein enzyme discovered in 1940, by Abraham and Chain,[1] was suggested as a possible approach to the treatment of penicillin reactions by Becker[2] in 1956. He reported that penicillinase administered intramuscularly in guinea pigs and human beings reduced the levels of previously administered penicillin to zero within one hour. Following a single dose of penicillinase, no circulating penicillin could be detected for periods of four to seven days, despite the continued injection of penicillin twice a day. Chen and co-workers,[3] studying the effects of penicillinase in rabbits, reported that it is rapidly absorbed, reaches its maximum activity about one hour after intramuscular injection, and is slowly metabolized, remaining active for a prolonged period. A dose of 5,000 units of penicillinase was found to inactivate 100,000 units of either crystalline or procaine penicillin G. While these workers failed to anaphylactically sensitize a small number of guinea pigs, Fisher and associates,[4] in a much more extensive immunologic study, demonstrated that penicillinase is a very strong antigen. They found that all guinea pigs and rabbits in their series became sensitive to penicillinase when given repeated injections alone or in combination with human albumin. Freund's adjuvant potentiated this sensitizing activity of penicillinase.

A purified form of penicillinase was used by a number of clinical investigators, who reported favorably on its effectiveness in controlling the manifestations of penicillin allergy.[5-7] In view of the allergist's great interest in the problem of penicillin sensitivity, a clinical study was undertaken by the Committee on Drugs of the American Academy of Allergy approximately one year ago.[8] During this time a total of fifty case reports of patients treated with penicillinase have been submitted by the following members of the Committee: Clarence Bernstein, Orlando, Florida; Theodore B. Bernstein, Evanston, Illinois; Joseph Besser, Philadelphia, Pennsylvania; Earl B. Brown, New York, New York; Monroe Coleman, Stamford, Connecticut; Seymour B. Crepea, Madison, Wisconson; Alex S. Friedlaender, Detroit, Michigan; Sidney Friedlaender, Detroit, Michigan; Frank T. Joyce, Denver, Colorado; Lloyd D. Mayer, Lexington, Kentucky; Gustavus A. Peters, Rochester, Minnesota; Isadore Pitesky, Long Beach, California; Samuel E. Rynes, Philadelphia, Pennsylvania; Aaron D. Spielman, New York, New York; and Paul VanArsdel, Jr., Seattle, Washington.

This report is based on studies by the Committee on Drugs of the American Academy of Allergy.

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*Chairman, Committee on Drugs, American Academy of Allergy.

MATERIALS AND METHODS

Penicillinase* was supplied in the form of 800,000 unit ampules of a lyophilized powder to be dissolved in 2 c.c. of sterile distilled water at the time of use. The recommended dose was 800,000 units intramuscularly, repeated in three days if necessary. The manufacturer's brochure, however, mentioned that the solution might be given intravenously if the urgency of the situation warranted.

Each investigator was advised to use penicillinase in any case which, in his judgment, was one of penicillin allergy. Special forms were prepared for the accumulation of the desired information in a uniform manner, and a report on every patient treated was submitted for analysis. Each of the clinicians involved in this study was intimately concerned with the treatment of allergic reactions and well aware of the vagaries of allergic symptomatology and therapy, the possibilities of spontaneous recovery, and the difficulties of accurately relating the symptom complex under consideration to a particular antigen (in this case, penicillin). In some instances, the investigator was the first physician to render treatment; in others, he was called in consultation because of his special interest in such cases and usually because the patient was not progressing satisfactorily. Because of the diverse manifestations of penicillin allergy and the variation from patient to patient, each case report was carefully studied and placed into a generally accepted classification. The fifty case reports submitted may be categorized as follows:

Type	*Number of cases*
Serum sickness type	19
Acute urticarial eruptions	13
Acute nonurticarial eruptions	4
Chronic urticarial eruptions	9
Acute anaphylactic reactions	5
Total	50

CLINICAL OBSERVATIONS

In attempting a fair evaluation of the effects of penicillinase in the cases submitted, it appears desirable to consider separately the various types of reaction involved and the possible influence of penicillinase on their course.

Reactions of Serum Sickness Type.—Nineteen patients manifested symptoms of urticaria, fever, and joint involvement from three to fourteen days after the administration of penicillin. Treatment with penicillinase was instituted from one to thirty days after the onset of symptoms.

In eleven cases complete clearing of symptoms occurred in ten to ninety-six hours after the use of penicillinase. Lasting improvement occurred in six instances after a single injection. Two improved temporarily after one

*Penicillinase (Neutrapen brand) used in this study was generously supplied by B. M. Lanman, M.D., and William T. Strauss, M.D., SchenLabs Pharmaceuticals, Inc., New York, New York.

injection with subsequent recurrence but cleared permanently after a second injection. Three cases showed improvement only after a second injection had been given.

In two of those who improved penicillinase was the only treatment given. In two others improvement occurred only after penicillinase was added to existing treatment. In the remaining seven cases penicillinase was used concomitantly with other measures, such as steroids, ACTH, and antihistaminics.

Eight patients failed to improve in direct relationship to penicillinase administration which was given from one to nine days after onset of symptoms. Five of these received only one injection; two were given two injections; and one received three injections without favorable response. One of these patients had also received a local anesthetic, a blood transfusion, and iodides, in addition to penicillin, before the development of the serum sickness type of reaction.

Acute Urticarial Eruptions.—Thirteen patients developed acute urticarial eruptions within minutes to five days after penicillin administration. Ten were given penicillinase within the first twenty-four hours; three received such treatment two to six days later.

In seven patients the symptoms cleared completely within one to seventy-two hours after a single injection of penicillinase. All of these patients received epinephrine injections and antihistaminics in addition to penicillinase. However, in three cases symptoms had been present for two to six days and cleared permanently only after penicillinase was added to treatment. In one other case, clearing of urticaria began in one hour after administration of penicillinase but recurred several hours later. No additional injections of penicillinase were given in this case.

Five patients in this category failed to improve after penicillinase, although each received an injection within twenty-four hours following onset of symptoms. Four of these received only one dose of penicillinase, while one was given a second dose without apparent benefit.

Acute Nonurticarial Eruptions.—Four patients developed morbilliform, erythematous, or "erythema multiforme type" eruptions within twenty-four hours of penicillin administration. Penicillinase was given from four to eight days after onset. The eruption in one case cleared permanently one hour after penicillinase, whereas previous treatment with antihistaminics had failed to benefit. In three other patients intensive use of steroids or ACTH and antihistaminics accompanied the administration of penicillinase. Complete clearing of the eruption was reported in two to four days in these three cases.

Chronic Urticarial Eruptions.—Nine patients had recurrent urticaria and angioneurotic edema, which had its inception in relation to penicillin administration, of one to twelve months' duration. One patient cleared permanently within a short time following a single injection of penicillinase. Another patient cleared temporarily after a single dose but improved and remained well only after a second dose had been given. In two patients

temporary improvement lasting up to two weeks was observed after two injections of penicillinase. In one of these, penicillinase was not repeated when symptoms recurred. In the other, a repetition of the same treatment failed to benefit. Still another patient was reported slightly improved after penicillinase, but complete clearing was not evident.

Four patients in this category failed to achieve any discernible benefit from penicillinase administration. Each had received two doses of penicillinase at the recommended intervals.

Acute Anaphylactic Reactions.—Four patients who experienced reactions of the anaphylactic type, consisting of shock, bronchoconstriction, and angioedema, within one hour following penicillin administration received penicillinase. Other measures for the treatment of shock and bronchoconstriction were administered in each instance.

In one patient penicillinase was given after thirty-six hours of intensive steroid and antihistaminic therapy had failed to control angioedema. Some improvement was noted shortly after the first injection of penicillinase, with further improvement and subsequent clearing after a second dose three days later.

Three patients received penicillinase immediately following the onset of their reaction in addition to other emergency measures. In two of these, complete recovery ensued. In the third case, severe urticaria was still present at the end of twenty-four hours and was treated and controlled with steroids. A second injection of penicillinase was not given.

SIDE EFFECTS

Pain at the site of injection was reported in the majority of cases. In addition, the occurrence of local swelling and tenderness, sometimes very severe, for several days afterward was reported in twenty-two patients. The occurrence of an acute febrile reaction within a short time following the administration of penicillinase was noted in thirteen patients. In several instances this was quite alarming to the attending physician, and a second dose of penicillinase which otherwise would have been given was deemed inadvisable.

COMMENT

It would be most difficult, if not foolish, to attribute each instance of improvement noted in the aforementioned series to the use of penicillinase. In many cases other potent therapeutic agents were used concomitantly. Even in those cases where penicillinase was used alone, it is difficult to be absolutely certain. Any clinician familiar with the variable course of allergic reactions, especially those related to penicillin hypersensitivity, realizes that a much greater experience with penicillinase is necessary before its exact value can be properly assessed. One investigator in this study treated every other penicillin reaction encountered in his institution with penicillinase. Three cases treated with penicillinase showed improvement within

a few days of its administration. In two other similar patients with reactions of the serum sickness type who did not receive the enzyme, one improved spontaneously forty-eight hours after onset, and in another symptoms which had persisted for three weeks subsided four days after hospitalization without specific treatment.[9] Indeed, others have reported good response in such cases even after placebo treatment.[5]

One cannot help being impressed, however, by the rapid improvement which occurred following the administration of penicillinase in some reactions of the serum sickness type and urticarial eruptions. While most of the acute reactions treated are self-limited, the rapidity of clearing which occurred in some instances shortly after the enzyme was used is sufficiently striking to suggest that it was of definite therapeutic benefit. A permanent improvement in chronic urticarial eruptions, if it occurs, would seem equally impressive, especially when other treatment measures have failed to influence the course of such symptoms.

From the experimental work with penicillinase, there is little reason to believe that it has a place in the treatment of the acute anaphylactic type of reaction. While it is possible that it may be useful in controlling the later symptoms of this sensitivity reaction, it cannot take the place of the emergency lifesaving measures necessary to overcome shock and relieve bronchospasm. A more rapid effect in neutralizing circulating penicillin might be achieved by giving penicillinase intravenously, but the foreign protein type of reaction so frequently encountered with intramuscular administration would make this route seem ill-advised. In the present series, one patient did receive penicillinase intravenously, with a subsequent shaking chill and a frightening febrile reaction.

In considering those who failed to improve following the use of penicillinase, a number of explanations must be considered. First of all, there is the possibility that the sensitivity reaction was due to something other than penicillin. It was noted in more than one patient in this series who failed to benefit that other drugs were used at the same time as penicillin. This line of reasoning might be carried much farther by saying that if a patient fails to respond to penicillinase, the cause of his difficulty is either not due to penicillin allergy or there are new or continued contacts with penicillin antigen from less obvious sources.

Next, in the case of treatment failure, there is also the possibility that the patient failed to receive enough penicillinase to neutralize the residual penicillin. It was noted in a number of cases in the present study that substantial and prolonged improvement did not occur until a second injection of penicillinase was given. It would therefore seem reasonable that a second dose should be administered if the patient fails to respond favorably to the first, before it is concluded that penicillinase is ineffective.

In view of the relative ease of sensitization to penicillinase in animals reported by Fisher and co-workers,[4] the possible effects of readministration of penicillinase to human beings must be given serious consideration. While

presumably a patient who experiences a penicillin reaction would in the future not be given penicillin and therefore would not require penicillinase again, there are sufficient possibilities that penicillin might be given inadvertently or encountered in some overt form, making it desirable to administer the enzyme again in treatment. The possibility of anaphylactic or other allergic reactions to penicillinase itself could then pose a real problem. This type of occurrence could become even more frequent if the recommendation that penicillinase be given prophylactically to any patient with a history of penicillin allergy who is to be given a vaccine, such as polio or adenovirus vaccine, known to contain penicillin were to be followed. Until such time as the sensitizing potential of penicillinase in human beings is further investigated and ascertained, the advisability of routine administration of this protein enzyme in such cases is open to question. One investigator who submitted cases for this report tested three patients with penicillinase before and two weeks after its administration. All gave a negative cutaneous response before treatment, while two weeks afterward the skin test reaction to the enzyme became strongly positive. Three other patients who were tested after receiving penicillinase also reacted strongly, while three control patients were negative.[10]

Since the conclusion of this clinical study, we have learned that a highly purified form of penicillinase has been developed which may have less local and systemic effect, and possibly lower antigenicity.[11]

SUMMARY

A purified form of penicillinase was used in treatment of fifty cases of sensitivity reactions related to the administration of penicillin. Rapid improvement following intramuscular administration was observed in some cases of reactions of the serum sickness type, acute urticarial and nonurticarial eruptions, and chronic urticaria. A number of similar cases failed to improve with the same treatment. Two injections of 800,000 units each at three-day intervals appeared more effective than a single dose. Its mode of action is such that it does not appear to have a place in the treatment of immediate anaphylactic reaction. Local pain and swelling and systemic febrile reactions were commonly encountered after intramuscular injections of the enzyme. Intravenous use is not advised. The antigenicity of penicillinase is such that the possibility of sensitization from repeated administration must be considered in its use.

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10300 WEST SEVEN MILE RD.

Erratum

In the article entitled "A Quantitative Study on the In Vitro Release of Histamine From Leukocytes of Atopic Persons" by VanArsdel, Middleton, Sherman, and Buchwald, which appeared in the September, 1958, issue of the JOURNAL, the concentrations reported in the seventh and eighth lines of the second paragraph on page 435 should be corrected to read as follows:

1.6×10^{-9}, 1.2×10^{-11}, and 1.4×10^{-8}.

Obituaries

J. HARVEY BLACK

ON NOV. 30, 1958, J. Harvey Black talked to his Sunday School class at the Highland Park Presbyterian Church in Dallas. This class had been one of his great joys for many years. He spoke of the blessings which we as Americans enjoy and mentioned briefly a number of the good things which had come to him throughout his life. He concluded with a quotation from the 16th Psalm: "The lines are fallen unto me in pleasant places: yea, I have a goodly heritage." He sat down in a nearby chair and died almost instantly.

Dr. Black received his medical education at the Southern Methodist College, where he later became professor of pathology and bacteriology and served as dean in 1914 and 1915. After this school was discontinued, he held professorial rank in pathology, bacteriology, preventive medicine, and clinical medicine at Baylor University School of Medicine, and since 1942 he had been a professor at Southwestern Medical School.

He became a Fellow of the American Association for the Study of Allergy in 1924 and was its president in 1935. He was president of the American Society of Clinical Pathologists in 1929-1930. Since 1945 he had been editor of the Allergy Section of *Biological Abstracts,* and he was a past member of the editorial board of the JOURNAL OF ALLERGY. He was a Fellow of the American College of Physicians, the New York Academy of Science, the American Public Health Association, and the American Society of Clinical Pathologists.

In addition to carrying on a large private practice, he found the time and energy to write many papers in the fields of bacteriology, pathology, and allergy and to complete the monumental task of revising Vaughan's *Practice of Allergy* (published by The C. V. Mosby Company, St. Louis) for its second and third editions. Just prior to his death, he had completed a revision of the "Section on Allergy" in Tice's *System of Medicine* (published by W. F. Prior Company, Hagerstown, Md.).

Dr. Black's interests and activities extended beyond the boundaries of medical science. He was deeply concerned with the racial problems of his city and of the South. For many years he was chairman of a civic committee which worked for the betterment of interracial relations. It appealed greatly to his sense of humor when a Dallas paper reported: "Dr. Black, a white man, introduced Dr. White, a black man," as the speaker at one of the meetings sponsored by this committee. He was a devoted Rotarian. The Rotary creed—"He profits most who serves best"—fitted his every action and deed. The Dallas Rotary Club has established a living memorial to him in the form of the J.

Harvey Black Memorial Fund, the proceeds of which will be used for such worthy purposes as scholarships for deserving students, aid for crippled and retarded children, etc.

As a greatly respected member of the Academy and the other medical societies to which he belonged, and as an elder of his church, he was asked to serve on committees which dealt with many thorny problems. His associates on such committees testify that he was a stabilizing influence and always softened any criticism with a kind word. His tact and marvelous sense of humor often relieved the tension which sometimes arose in the discussion of controversial subjects.

Because of his poise and keen wit, he was frequently asked to serve as a toastmaster. His witticisms were always kindly, never coarse, and invariably barbless. He had a beautiful tenor voice and sang in his church choir. Naturally, he was asked to sing at funerals. It was not generally known that he gave his services as freely at the funerals of paupers as he did for the well-to-do. Nor were his loans to needy students and his many kind deeds for others known to any but the recipients.

Because we in this Academy feel his loss so deeply, we can realize and deeply sympathize with the greater loss to his devoted and close-knit family.

The older members of the Academy recall the epic paper, "The State of Allergy," which Dr. Black read at a joint meeting of the parent societies in 1935. In this he presented in his inimitable humorous way the foibles of the allergists. He ended with the sentence: "In spite of all I have said, for my fellow allergists I have a great respect and much affection." This feeling was returned to him manyfold by all who knew him.

The world is a better place in both a spiritual and a scholarly sense because Harvey Black lived and worked in it.

S. S. B.

T. HARRY SAFERSTEIN

T. HARRY SAFERSTEIN, of St. Joseph, Missouri, died on July 28, 1958, of an acute recurrent coronary lesion.

He was born in St. Joseph, on Jan. 30, 1906, and he obtained his preliminary education there. He received his doctor of medicine degree from the Kansas University School of Medicine in June, 1930. He interned at Broadlawns Hospital, Des Moines, Iowa, and subsequently was a resident at Menorah Hospital, Kansas City, Missouri.

He served as a lieutenant commander in the Navy during World War II, at which time he suffered his first coronary lesion.

Dr. Saferstein was a member of the Buchanan County Medical Society, the Academy of Allergy, the College of Allergy, and the Rotary Club.

Dr. Saferstein was a keen and sincere student of allergy, the practice of which he carried out with infinite and exacting detail. Not only a large group of friends but also a host of appreciative patients mourn his death.

H. J. R.

Announcement

Lewis Webb Hill Honored by Allergy Group

Dr. Lewis Webb Hill of Boston, Massachusetts, was honored by the Section on Allergy of the American Academy of Pediatrics at a meeting held in Chicago, Illinois, on Oct. 19, 1958. Dr. Arthur J. Horesh of Cleveland, Ohio, national chairman of the Section, presented Dr. Hill with a scroll which read as follows:

> By unanimous vote of the Section on Allergy of the American Academy of Pediatrics assembled in Chicago, Illinois, at the Twenty-Seventh Annual Meeting of the Academy, this Scroll is awarded to
>
> LEWIS WEBB HILL, M.D.
>
> as an expression of gratitude and appreciation for his untiring services as a pioneer investigator in advancing our knowledge of allergy in pediatrics, particularly in the difficult field of allergic skin diseases.
>
> For his brilliant teaching of pediatric allergy at Harvard Medical School and in this Academy for many years.
>
> For the Section:
>
> Harry L. Mueller, M.D.
> Jerome Glaser, M.D.
> Arthur J. Horesh, M.D.,
> Chairman
>
> This 21st day of October, 1958.

Books Received

The Author-Publisher-Printer Complex. By Robert S. Gill. Baltimore, 1958, Williams & Wilkins Company. 134 pages. Price, $2.25.